

Development of a biological process for surplus electricity conversion to biogas via hydrogen

Von der Fakultät für Ingenieurwissenschaften, Abteilung
Bauwissenschaften der Universität Duisburg - Essen zur Erlangung des
akademischen Grades

Doktor-Ingenieur

genehmigte Dissertation

vorgelegt von

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Eingereicht: 04.10.2017

Mündliche Prüfung: 27.02.2018

UNIVERSITÄT DUISBURG-ESSEN
SIEDLUNGSWASSERWIRTSCHAFT UND
ABFALLWIRTSCHAFT

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Essen, Germany
2017

Acknowledgements

The present work is the result of a Marie Curie scholarship in the framework of the ATBEST (Advanced Technologies for Biogas Efficiency, Sustainability and Transport) program. It was hosted for three years at the Department of Urban Water and Waste Management, University of Duisburg – Essen.

First of all, I would like to express my gratitude to Prof. Renatus Widmann for his advice, help and support but also for his understanding during these years.

I would like to also thank Thorsten Mietzel and Sebastian Schmuck for this collaboration.

To all the people of the 5th floor I extend my gratitude. To Ilka for her persistence on talking to me in German and for taking care of Annabelle. To Ruth for the advice and the breaks we did together. Of course to my office mate Jens for the lovely time including singing and basketball. I would particularly like to thank Sarah. Because of her help I had the most amazing apartment to enjoy and relax during these years.

Furthermore, I would like to thank all of the people at the laboratory who guided me and helped me. Special thanks go to Lukas and Leon.

I am particularly grateful for having my two girls, Erika and Asma, supporting me inside the university but mostly outside, during our everyday life. It was amazing having them as friends.

Now, since ATBEST was the greatest opportunity to learn, travel and meet new people, I had 14 great fellows and the best friend/‘not your mother’/ project manager, Simon Murray. All of them made even the presentations seem that are made between friends. Because of them, this experience was one of the greatest of my life.

I would like to also thank all of the supervisors of ATBEST program for their support, advice and feedback during the meetings. I would like to express special gratitude to Prof. Jerry Murphy for his valuable advice and help during my secondment in Ireland.

Finally, to all of the people I consider my family...σας αγαπάω.

This work was a part of ATBEST (Advanced Technologies for Biogas Efficiency, Sustainability and Transport) Marie – Curie Initial Training Network. This project was funded by the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n. 316838. ATBEST was coordinated by the QUESTOR centre at Queen's University Belfast (www.atbest.eu).

Table of Contents

Abstract	1
Abstrakt	2
1. Introduction	4
1.1 Thesis outline	5
2. Renewable energy sources – Renewable hydrogen production	6
2.1 Introduction	6
2.2 Surplus renewable energy.....	7
2.3 Renewable hydrogen production.....	8
2.3.1 Fundamentals of electrolysis	8
2.3.2 Types of electrolysis technology.....	9
3. Anaerobic digestion	14
3.1 Basic principles of anaerobic digestion	14
3.2 Microbiology and metabolic pathways of anaerobic digestion.....	15
3.2.1 Hydrolysis	16
3.2.2 Acidogenesis	17
3.2.3 Acetogenesis	18
3.2.4 Methanogenesis	20
3.3 Factors affecting anaerobic digestion process	22
3.4 Biogas upgrading methods.....	26
3.4.1 Humidity removal.....	27
3.4.2 Desulphurization of biogas	27
3.4.3 CO ₂ removal	28
3.4.3.1 Absorption technology	29
3.4.3.2 Pressure swing adsorption (PSA).....	30
3.4.3.3 High – pressure membrane separation	31

3.4.3.4 Cryogenic upgrading	32
3.5 Power to Gas concept – Hydrogen utilization in anaerobic digestion process ...	32
3.5.1 Catalytic methanation	34
3.5.2 Biological methanation.....	35
3.5.2.1 In – situ biological methanation	35
3.5.2.2 Ex – situ biological methanation	36
4. Materials and methods – Experimental configuration	37
4.1 Wastewater characteristics and feedstock preparation	37
4.2 Experimental configuration.....	38
4.3 Analytical methods	40
4.3.1 Total and volatile solid concentration.....	41
4.3.2 Chemical oxygen demand	41
4.3.3 Volatile fatty acids.....	43
4.3.4 Ammonium and phosphorous concentration	44
4.3.5 Biogas volume and composition	44
4.3.6 Fluorescence in situ hybridization (FISH)	45
4.4 Theoretical estimation of the methane potential.....	47
4.4.1 Biomethane Potential Test (BMP).....	47
4.4.2 Buswell's formula.....	49
4.5 Reactor start – up.....	49
5. Results and discussion	51
5.1 Introduction	51
5.2 Theoretical maximum biomethane potential (BMP_{th}) and substrate chemical oxygen demand equivalent (COD_{th})	51
5.3 Biomethane Potential Test Performance.....	53
5.4 Performance of the 1 st experimental system ($CSTR_1$).....	53
5.4.1 Phase #1	54

5.4.2 Phase #2	55
5.4.3 Phase #3	56
5.4.4 Phase #4	57
5.4.5 Phase #5	59
5.4.6 Phase #6	60
5.4.7 Overall performance of experimental system CSTR ₁	62
5.5 Performance of the 2 nd experimental system (CSTR ₂)	64
5.5.1 Phase #1	65
5.5.2 Phase #2	66
5.5.3 Phase #3	67
5.5.4 Phase #4	68
5.5.5 Change of metabolic products and pH value	70
5.5.6 Overall performance of experimental system CSTR ₂	73
5.6 <i>Ex – situ</i> upgrading experimental system CSTR ₃	74
5.7 Anaerobic digestion pathway through FISH analysis	79
5.8 Comparison of performance of all experimental configurations	83
5.9 Comparison of performance with other reported systems	88
6. Concluding remarks and outlook	92
7. References	95

Table of Figures

Figure 1: Share of EU energy production by source, 2015 (Source: Eurostat) http://ec.europa.eu/eurostat/cache/infographs/energy/bloc-2b.html	6
Figure 2: Basic schematic configuration of an alkaline electrolysis cell	10
Figure 3: Basic schematic configuration of polymer electrolyte membrane	11
Figure 4: Basic schematic configuration of solid oxide electrolysis.	12
Figure 5: Schematic representation of the anaerobic digestion process.	16

Figure 6: Effect of H_2 partial pressure on reactions involved in interspecies H_2 transfer during oxidation of ethanol, propionate and butyrate coupled to methanogenesis (Zinder 1993).	20
Figure 7: pH and temperature influence on NH_3 dissociation (Angelidaki et al., 2003).	25
Figure 8: Different pathways of power to methane concept at a biogas plant.	33
Figure 9: Schematic configuration of the 1 st experimental system (CSTR ₁).	39
Figure 10: Schematic configuration of the 2 nd experimental system (CSTR ₂).	39
Figure 11: Main units of the system AMPTS II, Bioprocess Control AB, Sweden. ...	48
Figure 12: Schematic indication of the sampling points on the configuration.	54
Figure 13: Performance of CSTR ₁ system during start-up and phase #1. Time evolution of biogas and CH_4 production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).	55
Figure 14: Performance of CSTR ₁ system until the 2 nd operational phase. Time evolution of biogas and CH_4 production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).	56
Figure 15: Performance of CSTR ₁ system until the 3 rd operational phase. Time evolution of biogas and CH_4 production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).	57
Figure 16: Performance of CSTR ₁ system until the 4 th operational phase. Time evolution of biogas and CH_4 production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).	58
Figure 17: Thick layer created during operational phase 5 at CSTR ₁ system.....	59
Figure 18: Overall performance of CSTR ₁ system until the 5 th operational phase. Time evolution of biogas and CH_4 production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).....	60
Figure 19: Performance of the CSTR ₁ system. Time evolution of biogas and CH_4 production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).....	61

Figure 20: Time evolution of biogas and CH ₄ production rate per g VS fed during each operational phase of system CSTR ₁	63
Figure 21: Ceramic diffusers for hydrogen addition.....	64
Figure 22: Performance of system CSTR ₂ during start-up and phase #1. Time evolution of biogas and CH ₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).	66
Figure 23: Performance of the system CSTR ₂ during 2 operational phases. Time evolution of biogas and CH ₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).	67
Figure 24: Performance of the system CSTR ₂ during 3 operational phases. Time evolution of biogas and CH ₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).	68
Figure 25: Overall performance of system CSTR ₂ . Time evolution of biogas and CH ₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).....	69
Figure 26: Time evolution of average VFA concentration and biogas production rate of system CSTR ₂	70
Figure 27: Composition of biogas and pH values during each operational phase of system CSTR ₂	72
Figure 28: Time evolution of biogas and CH ₄ production rate per g VS fed during each operational phase of system CSTR ₂	74
Figure 29: Schematic configuration of the 3 rd experimental system (CSTR ₃).....	75
Figure 30: Methane composition and volumetric productivity of CSTR ₃	76
Figure 31: Homoacetogenesis metabolic pathway.	77
Figure 32: Combination of probes ARCH915 (Cy-3) and EUB338 (6-FAM). Left side: merged channels, Right side: detected Archaea.....	81
Figure 33: Left side: Typical morphology of Methanosaetaceae (filaments) detected with probe ARCH915 (6-FAM) and MSMX860 (Cy-3), Right side: Typical morphology of Methanobacteriaceae (filamentous rods) detected with probe MB1174 (Cy-3)....	82

Figure 34: Comparison of methane production rate of the theoretical yield (BMP_{th}), the biomethane potential test (BMP), the control reactor (Control) and the CSTR₂ system..... 84

Figure 35: Average value of the main biogas components for all three experimental configurations..... 87

List of Tables

Table 1: Typical characteristics of different electrolysis technologies (modified from Persson et al., 2014, Götz et al., 2016)..... 13

Table 2: Standard Gibbs free energy of certain volatile fatty acids metabolic reactions through syntrophy..... 19

Table 3: Characterization of substrate and inoculum 37

Table 4: Modified composition of the trace metal solution (Hussy et al., 2004)..... 38

Table 5: Operational conditions of gas chromatograph during the analytical determination of VFAs..... 43

Table 6: Analytical methods for the determination of sCOD, $NH_4^+ - N$ and $PO_4^{3-} - P$ concentration..... 44

Table 7: Composition of the hybridization buffer at the applied formamide concentrations..... 46

Table 8: Composition of the washing buffer corresponding to the formamide concentrations applied during the hybridization. 47

Table 9: Theoretical calculation of maximum methane potential and methane concentration using the Buswell Equation (based on Murphy et al., 2013). 52

Table 10: Theoretical calculation of COD equivalent expressed in g COD g VS⁻¹. ... 52

Table 11: Average values of the main parameters during each operational phase of system CSTR₁..... 62

Table 12: Summary table of the average values of the main parameters during each operational phase of system CSTR₂. 73

Table 13: Main characteristics of methanogenic order..... 79

Table 14: Oligonucleotic probes used and their specificity.....	80
Table 15: Average values of the main parameters for all three experimental configurations.....	86
Table 16: Reported systems and performance data.	91

Abbreviations and Symbols

AD	anaerobic digestion
AEC	alkaline electrolysis cell
AgSO ₄	silver sulphate
BMP	biomethane potential
BPR	biogas production rate
CH ₃ COOH	acetate
CH ₄	methane
CHP	combined heat and power
CO ₂	carbon dioxide
COD	chemical oxygen demand
CSTR	continuously stirred tank reactor
DAQ	data acquisition
DMEA	di-methyl ethanol amine
EC	electrical conductivity
EU	European Union
FAS	ammonium iron (II) sulphate hexahydrate
FID	flame ionization detector
FISH	fluorescence in situ hybridization
GHG	greenhouse gas
H ⁺	hydrogen ions
H ₂	hydrogen
H ₂ O	water
H ₂ S	hydrogen sulphide
He	helium

HFM	hollow fiber membrane
HgSO ₄	mercury (II) sulphate
HRT	hydraulic retention time
K ₂ Cr ₂ O ₇	potassium dichromate
KOH	potassium hydroxide
LCFA	long chain fatty acids
MEA	mono ethanol amine
MPR	methane production rate
N ₂	nitrogen
NaOH	sodium hydroxide
NDIR	nondispersive Infrared
OLR	organic loading rate
PEM	polymer electrolyte membrane
PSA	pressure swing adsorption
PtG	Power to Gas
PV	photovoltaics
RES	renewable energy sources
S	sulphur
sCOD	soluble chemical oxygen demand
SiO ₂	silica gel
SOEC	solid oxide electrolysis cell
TS	total solids
TSS	total suspended solids
UASB	upflow anaerobic sludge blanket
UK	United Kingdom
VFAs	volatile fatty acids
VS	volatile solids
VSS	volatile suspended solids
WWTP	wastewater treatment plant

Abstract

Wind and solar energy have a vital role to play as promising renewable energy sources. However, energy demand and consumption varies thus, making these energy sources subject to both seasonal as well as hourly variation. In times of overproduction a high percentage of wind or solar energy is judged to be a surplus. The Power – to – Gas (PtG) concept can contribute so as amounts of renewable energy are not wasted, but are rather used.

The PtG concept combines the excess generated electricity from renewable sources with the anaerobic digestion process. Surplus electricity can be converted to methane via hydrogen production by water electrolysis and then hydrogen utilization for biological methanation.

The present work focuses on biological methanation with hydrogen utilization in the anaerobic digestion process. Different experimental systems were installed and operated in a continuous way.

The supply of hydrogen to the anaerobic digester for *in – situ* biogas upgrading as well as implementation of an *ex – situ* biogas upgrading process for hydrogen and carbon dioxide utilization, as the sole feeding stream, were examined.

In this study, different CSTR reactors, treating sewage sludge, were evaluated. The experiments were conducted at mesophilic temperature. Hydrogen was injected. Various operational conditions were implemented. The treatment system's performance was monitored. The effects of different hydrogen flow rates on the process performance were monitored. For further improvement of methane yield, biogas recirculation was tested.

High conversion efficiency was obtained. Methane production rate was increased compared to the control reactor. Methane content resulted in higher percentages both for *in – situ* and *ex – situ* processes.

The results suggest that hydrogen utilization into the anaerobic process can account for a possible way to deal with the high share of variable renewable power production. Surplus electricity can be converted to methane, an easily storable and grid compatible gas.

Abstrakt

Wind- und Solarenergie spielen eine gewichtige Rolle als erfolgsversprechende erneuerbare Energiequellen. Der Energiebedarf und der Energieverbrauch sind jedoch variabel, deshalb unterliegen diese Energiequellen stündlichen und jahreszeitlichen Schwankungen. In Zeiten der Überproduktion, kann ein hoher Anteil der Wind- und Solarenergie, als Überschuss betrachtet werden. Das Power to Gas Prinzip (kurz *PtG*, kann mit „Elektrische Energie zu Gas“ übersetzt werden) kann dazu beitragen, dass die überschüssige Energie nicht verschwendet, sondern gespeichert wird.

Bei dem *PtG* Prinzip, wird der Überschuss der erneuerbaren Energie, mit der anaeroben Vergärung kombiniert. Stromüberschuss kann in Methan umgewandelt werden. Dies geschieht durch Wasserstofferzeugung mittels Wasserelektrolyse und durch die anschließende Verwendung von Wasserstoff für die biologische Methanisierung.

Die vorliegende Arbeit legt den Schwerpunkt auf die biologische Methanisierung mit der Verwendung von Wasserstoff in der anaerobe Vergärung. Verschiedene Versuchssysteme wurden installiert und fortlaufend betrieben.

Die Einspeisung von Wasserstoff in den anaeroben Fermenter einer *in – situ* Biogasaufbereitung und ein *ex – situ* Prozess für die Biogasaufbereitung, in dem ausschließlich Wasserstoff und Kohlendioxide zugegeben wurden, wurden untersucht.

Im Rahmen dieser Arbeit, wurden verschiedene CSTR Reaktoren, in denen Klärschlamm behandelt wurde, untersucht. Die Experimente fanden unter mesophilen Bedingungen statt. Wasserstoff wurde hinzugeführt. Verschiedene Betriebsbedingungen wurden umgesetzt. Die Leistung des Behandlungssystems wurde überwacht. Die Wirkungen von verschiedenen Wasserstoffdurchflussmengen auf die Effektivität des Prozesses wurde ebenfalls überwacht. Zur weiteren Optimierung des Methanertrags, wurde eine Rückführung des Biogases untersucht.

Ein hoher Umwandlungswirkungsgrad konnte erreicht werden. Die Produktionsrate von Methan stieg an, im Vergleich zu einem Kontrollreaktor. Sowohl beim *in – situ*, als auch beim *ex – situ* Prozess konnten höhere Methankonzentrationen beobachtet werden.

Die Ergebnisse zeigen, dass die Verwendung von Wasserstoff im anaeroben Prozess, eine Möglichkeit für die Verwendung von überschüssigen, erneuerbaren Energien darstellen kann. Stromüberschüsse können zu Methan umgewandelt werden und sind so einfach speicherbar.

1. Introduction

The European Commission has proposed a target of achieving 20% renewable energy in European Union's (EU) overall energy by 2020. Therefore, emerging renewable energy strategies come to the forefront. In realizing these targets bioenergy, wind and solar energy, as promising renewable energy sources, have a vital role to play.

Wind power was the energy technology with the highest capacity installations in 2016. According to Wind Europe organization, 12.5 GW of new wind power capacity was installed and grid-connected in the EU during 2016 (Nghiem A. & Mbistrova A., 2017). It accounted for 51% of all new installations. Solar photovoltaics (PV) came second with 6.7 GW (27%) and natural gas followed with 3.1 GW (13%). Germany was the largest market in 2016 in terms of wind energy annual installations, with 5,443 MW of new capacity. Germany remains the EU country with the largest installed wind power capacity, followed by Spain, the UK and France.

Energy derived from natural processes, such as sunlight and wind can be replenished faster than it is consumed. Solar and wind are fluctuating energy sources. Their output depends on availability of the primary source. An accurate prediction ahead of time is not possible thus, making these energy sources subject to both seasonal as well as hourly variation. Additionally, energy demand and consumption varies. In times of overproduction a high percentage of wind or solar energy is judged to be a surplus. In order for the potential to be fully utilized, the fluctuating and intermittent wind and solar energy sources have to be balanced for electricity grid stability purposes (Götz et al., 2016). The Power – to – Gas (PtG) concept can contribute so as amounts of renewable energy are not wasted, but are rather used.

The PtG concept combines the excess generated electricity with the anaerobic digestion process. Surplus electricity can be converted to an easily storable and grid compatible gas, methane (CH₄). Via a two-step process including: i) hydrogen production by water electrolysis and ii) hydrogen conversion to methane (biological methanation), the Power – to – Gas (or Power to Methane) system can account for biogas upgrading process.

1.1 Thesis outline

The present work focuses on the biological methanation part of a Power – to – Gas system. Biogas upgrading via hydrogen utilization is presented. The effect of hydrogen addition into an anaerobic reactor is experimentally evaluated both *in – situ* and *ex – situ*.

More specifically, the objectives of the research were:

- to supply hydrogen (H₂) to the organic waste feeding stream of an anaerobic digester aiming its conversion into methane
- modify the anaerobic configuration to ensure high hydrogen consumption rate
- optimize the operational conditions for hydrogenotrophic methanogens
- identify the methanogens facilitating the overall process
- evaluate the stability and robustness of the implemented systems

This research work is structured according to the following outline:

In Chapter 2, the fundamentals regarding renewable energy potential and renewable hydrogen production are presented.

The basic principles of anaerobic digestion are described in Chapter 3. The factors affecting the process performance and the metabolic pathways of anaerobic digestion are also indicated. Furthermore, biogas upgrading technologies are listed and key features of each discussed. Finally details on the different ways of methanation involved to a PtG concept are given.

In Chapter 4 materials and methods used for the continuous experiments are described. In addition, the experimental configurations of the implemented systems are indicated.

In Chapter 5, the performance of the evaluated systems is described in detail. A comparison is made between the implemented systems and the performance of the present work is compared to other research studies.

Finally, the main points and conclusions of this study are summarized in Chapter 6 while an outlook of the future energy scenery and suggestions for future research are also presented.

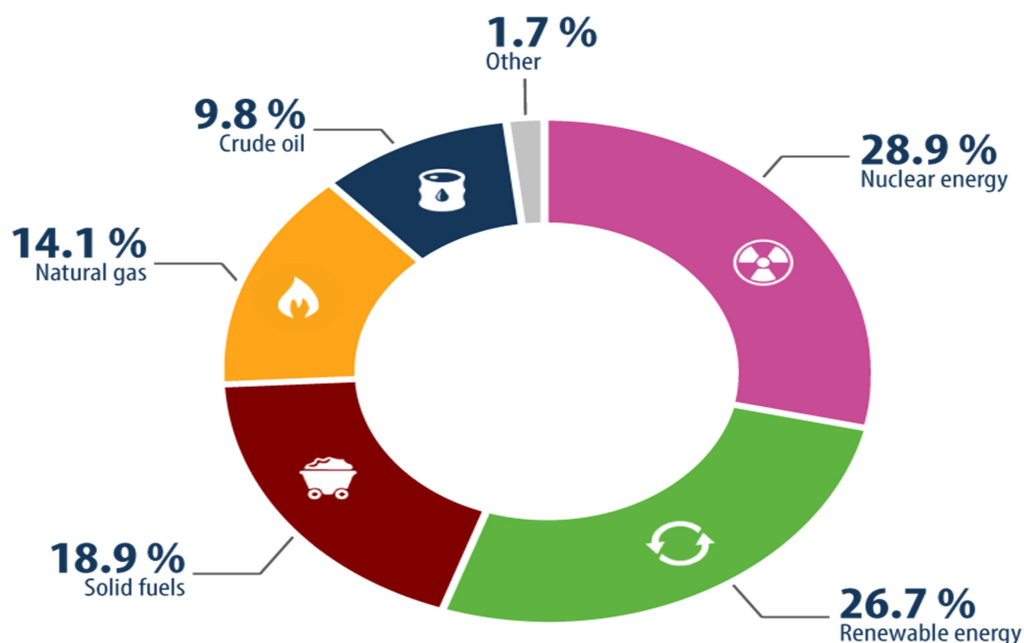
2. Renewable energy sources – Renewable hydrogen production

2.1 Introduction

Future energy roadmaps focus on a substantial rise of the share of renewable energy sources (RES). Solar power (thermal, photovoltaic), wind power, hydroelectric power, geothermal energy, tidal power, biofuels as well as the renewable part of waste are included in the renewable sources. The efforts to curtail the dependency on fossil fuels depend on the potential benefit deriving from renewable sources including greenhouse gas (GHG) emissions reduction.

According to Eurostat statistics within European Union (EU) the production of energy currently originates from a wide range of different energy sources. Among them are solid fuels (mainly coal), crude oil, natural gas, nuclear energy and renewable energy. The share of each source is given in Figure 1.

Share of EU energy production by source, 2015



Source: Eurostat

Figure 1: Share of EU energy production by source, 2015 (Source: Eurostat)
<http://ec.europa.eu/eurostat/cache/infographs/energy/bloc-2b.html>

Energy production varies from one EU-member to another. In total, however, renewable energy for the year 2015 was the second largest contributing source within EU (for EU-28, Eurostat).

In EU-28 for 2015, hydropower contributed as the largest source with a percentage of 14.4% of total primary energy production of renewable energy. While wind, solar and geothermal energy accounted for 12.7%, 6.4% and 3.1%, respectively, (Eurostat, 2015).

2.2 Surplus renewable energy

The amount of renewable electricity produced by technologies which vary their output due to weather conditions (i.e. solar and wind) is rapidly increasing and so is the need to develop suitable technologies to balance uneven electricity production and utilization (Persson et al., 2014).

Wind and solar energy technologies during the past years presented a high increase in new capacity installations. Despite this increase, there is a percentage of renewable energy that is not utilized due to varying wind conditions and electricity demand.

Wind turbines convert the kinetic energy of the wind into electricity. Both on land and offshore turbines are located around Europe. Wind speed determines the generated amount of power, thus making any power supply prediction over short time periods difficult.

Solar energy is also an infinite resource. The use of photovoltaic cell can convert the sunlight to electricity. Yet, solar energy is as well contingent on weather conditions.

The electricity production from these technologies is linked to fluctuations (Lund & Münster, 2003). Even though wind and solar energy are the backbone of the efforts made to limit global warming and create a decarbonized energy system, they are directly dependent on weather conditions and energy demand. Hence there are some obstacles yet to overcome.

Clear sky and windy weather can result in surplus electricity. According to Agora Energiewende, on May 2016, renewable energy plants in Germany generated over

87% of the country's total consumption. There are several countries experiencing this kind of energy surplus issue.

Incidents of excess electricity from renewable sources are no way a disaster. However, at times of overproduction, curtailment will be required. The appropriate infrastructure in order to respond in a timely manner to this kind of energy production is not yet accomplished. Conventional power plants, coal and nuclear plants should either stop or slow their energy production. But this is difficult to be implemented on time. An efficient way of adopting surplus electricity of renewable energy sources while minimizing the curtailment is required.

Combining excess electricity with two other energy production processes may be a possible way to deal with the high share of variable renewable power production. Producing renewable hydrogen via electrolysis is the first step for this combination.

2.3 Renewable hydrogen production

Hydrogen can be produced from the electric energy generated by wind and photovoltaic systems in different applications (Barbir 2005, Sherif et al., 2005). According to Ursúa et al., (2012) wind and PV systems are coupled with electrolyzers in order to produce hydrogen. The electrolysis system can be operated at the time of excess electricity, thus making the produced hydrogen completely renewable.

2.3.1 Fundamentals of electrolysis

Electrolysis is an electrochemical reaction, where direct electrical current is used to split water into its constituent elements, oxygen and hydrogen (Persson et al., 2014), according to Equation 1.



Electrolysis can be segmented into 2 steps (Götz et al., 2016). It yields to hydrogen and oxygen production in an electrochemical cell. The cell is consisted of two porous electrodes (anode and cathode), a membrane and an ionic – conductive electrolyte.

The reduction reaction occurs at the negatively charged cathode while the oxidation reaction takes place at the positively charged anode. An external circuit electrically connects the two electrodes. Direct electrical current is used. The charge difference results in water molecules ionization into hydrogen and oxygen ions. Hydrogen and oxygen are formed at the cathode and at the anode, respectively. Water electrolysis is an endothermic reaction hence, energy input is required. The most important parameters regarding electrolysis are efficiency, flexibility and lifetime (Götz et al., 2016).

2.3.2 Types of electrolysis technology

There are three different types of electrolysis technology, with the name of each technology taken from the electrolyte employed in the cell. The different types discussed here are:

- Alkaline electrolysis cells (AEC),
- Polymer Electrolyte Membrane (PEM),
- Solid Oxide Electrolysis Cells (SOEC).

Alkaline electrolysis cells (AEC) technology: is recognized as a mature technology (Ursúa et al., 2012). It constitutes the most extended technology at a commercial level worldwide (Kreuter & Hofmann 1998, Ivy 2004). As an electrolyte, an aqueous alkaline solution of potassium hydroxide (KOH) or sodium hydroxide (NaOH) is used. The operating principle of an AEC electrolyzer is illustrated in Figure 2.

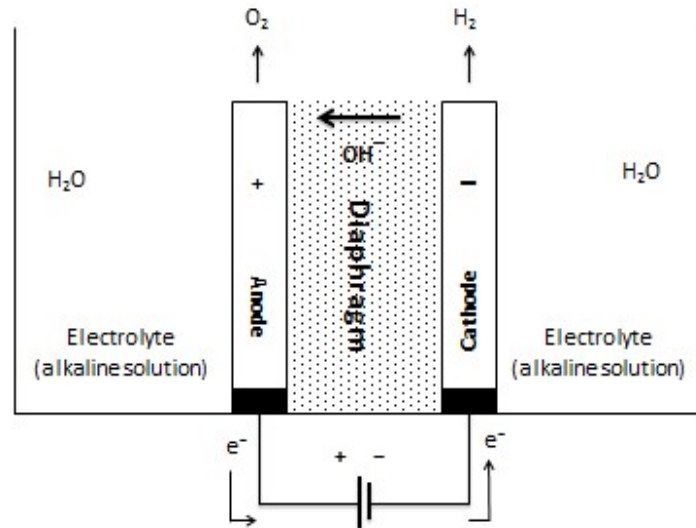
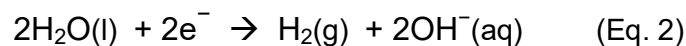
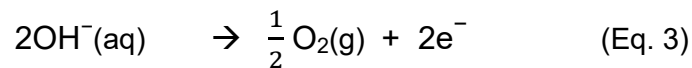


Figure 2: Basic schematic configuration of an alkaline electrolysis cell.

The cell is immersed in the liquid electrolyte and consists of two electrodes, with a gas tight diaphragm between them. Water is reduced at the cathode according to Equation 2.



Hydrogen gas and hydroxide anions are produced. The anions are circulated across the membrane. The established electric field (by an external power source) leads them to the anode. From there oxygen gas evolves according to Equation 3.



This technology operates either under atmospheric pressure or under higher pressure (up to 200 bar). AEC electrolyzers can operate between a wide range of their design capacity thus making it suitable combination with either a fluctuating or an intermittent power supply. The expected lifetime for an alkaline electrolyzer is about 30 years, which is high compared to the other types (Götz et al., 2016).

Polymer Electrolyte Membrane (PEM) electrolysis: This technology is also referred to as proton exchange membrane and solid polymer electrolyte (Takenaka et al., 2006, Millet et al., 2009, Ursúa et al., 2012). A solid polymer membrane is the electrolyte used. For water electrolysis the most commonly used membrane is Nafion®, DuPont™ membranes. The operating principle of a PEM system is shown in Figure 3.

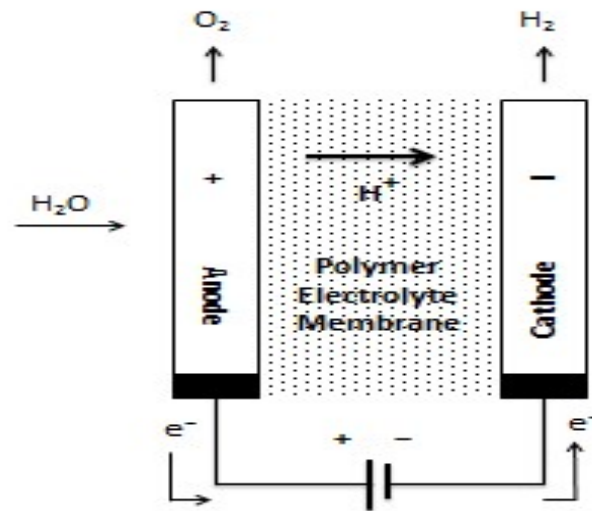
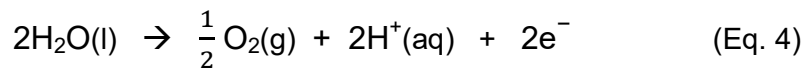
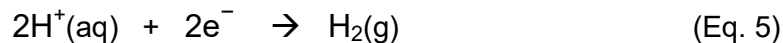


Figure 3: Basic schematic configuration of polymer electrolyte membrane.

According to the following equation (Equation 4) water is oxidized to produce oxygen at the anode.



Electrons and protons are also produced. Protons are circulated across the membrane. At the cathode they are reduced as Equation 5 illustrates.



The circuit is then closed and hydrogen is produced at the cathode. PEM electrolysis has the ability to operate at a low load of the rated capacity as well as at higher power density. Furthermore, they have the ability to work under variable power feeding regimes and respond quickly to power fluctuations (Ursúa et al., 2012). Thus, according to Carmo et al., (2013), PEM technology is well suited to variable wind and solar power. These conditions result in lower operation costs (Götz et al., 2016). However, due to the expensive membrane and the use of a noble metal as catalyst, the investment cost is high. The membrane needs to be exchanged every 5 to 10 years (Benjaminsson et al., 2013).

Solid Oxide Electrolysis Cells (SOEC): is the least mature technology. It is also known as high temperature electrolysis or steam electrolysis since significantly high operating temperatures (600 – 900° C) are required (Ursúa et al., 2012). The used

electrolyte is ceramic of yttria – stabilized zirconia (ZrO_2 doped with Y_2O_3). In Figure 4 the operating principle of this technology is indicated.

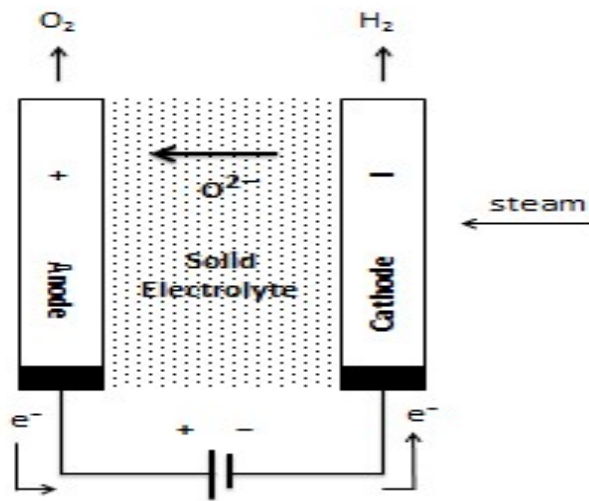
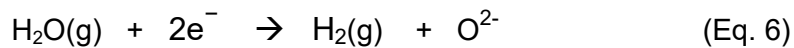
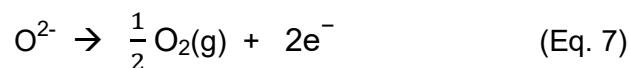


Figure 4: Basic schematic configuration of solid oxide electrolysis.

Steam is fed to the cathode and its reduction takes place. At the cathode, hydrogen production occurs according to the Equation 6.



At the cathode, oxide anions (O^{2-}) are also produced. These anions pass through the electrolyte. At the anode, they get recombined and oxygen is formed according to Equation 7.



The circuit is then closed by the released electrons.

In contrast to alkaline and PEM electrolyzers, here electrodes are in contact with a gas phase. Thus, the issue of maximizing the interfacial area in contact between the electrodes and the gaseous compounds is challenging (Berry 2004).

An available high temperature heat source can cover the amount of the required energy by supplied heat instead of electricity. The low electricity demand is considered to be the most significant advantage of SOEC technology. According to Benjaminsson et al., 2013, the electrochemical reactions occur at higher rates resulting in efficiency of 90 to 95% as compared to 60 – 70% for AEC and PEM

electrolysis. However, the fast material degradation, the limited long term stability due to the high operating temperature as well as the system's instability against fluctuating and intermittent power sources are reported as important challenges for the SOEC technology to overcome (Brisse et al., 2008, Laguna – Bercero 2012, Götz et al., 2016).

Table 1 summarizes the typical characteristics of the three different electrolysis technologies.

Table 1: Typical characteristics of different electrolysis technologies (modified from Persson et al., 2014, Götz et al., 2016).

	AEC	PEM	SOEC
State of development	commercial	commercial	laboratory
Type of electrolyte	alkaline solution	solid polymer membrane (Nafion)	ceramic of yttria – stabilized zirconia
Temperature (° C)	40 - 90	20 – 100	800 - 1000
Transient operation	suitable	suitable	not well suited
Renovation/Lifetime	up to 30 years	membrane exchange: 5 – 10 years	–
Efficiency	60 – 70%	60 – 70%	90 – 95%

Regarding the capital expenditures, AEC currently appears to be the most economical electrolysis technology. According to literature reports (Sterner & Stadler 2014, Götz et al., 2016) the investment for PEM systems is at least two times that of AEC systems. Limited information is available regarding SOEC systems. However, according to calculations carried out by Reytier et al., (2014) an estimated price will be comparable to the upper range of PEM price estimations.

Overall, hydrogen produced from electrolysis can then be employed in the anaerobic digestion process. This combination serves two purposes at the same time:

- Avoidance of the loss of excess renewable energy, and
- Improving the performance of anaerobic digestion.

3. Anaerobic digestion

Anaerobic digestion (AD) is a well – established biological process which results in organic pollution reduction and bioenergy production. The reason that anaerobic digestion process merits attention is based on the following two points:

- Via anaerobic digestion a high degree of degradation of the organic matter is achieved.
- The produced biogas can be used for energy production (electricity and heat) or to receive appropriate treatment in order to be used as a vehicle fuel (Angelidaki et al., 2003).

3.1 Basic principles of anaerobic digestion

The term anaerobic digestion refers to a biological process where organic carbon undergoes subsequent oxidations and reductions and is converted to its most oxidized (CO_2) and its most reduced (CH_4) state. This process is catalyzed by a wide range of microorganisms able to act within oxygen-free conditions. Through anaerobic degradation methane and carbon dioxide are mainly produced. Additionally, minor quantities of hydrogen, ammonia and hydrogen sulfide (typically less than 1% of the total volume of the gas) are generated. The mixture of these gaseous products is called biogas and the process is often termed as biogas process (Angelidaki et al., 2003). The reported advantages of anaerobic digestion are significant:

1. Anaerobic digestion is a suitable method for the processing of various agro-industrial wastes with a high organic load (Drosg et al., 2013).
2. High level of organic matter degradation can be accomplished (Gray et al., 2004).
3. The anaerobic microorganisms are characterized by relatively small biomass yield coefficient. Therefore, a small amount of the sludge is produced, 3 up to 20 times less than the aerobic processes (Gerardi et al., 2003).
4. Well adapted microorganisms are resistant to the absence of "food" for long periods, maintaining sufficiently their activity (Lettinga 1995).

5. With the use of biogas engines, the produced biogas enables the cogeneration of electricity and heat (combined heat and power, CHP) (Bitton et al., 2005).
6. The exploitation of the energy content (biogas) leads to a substantial reduction in the initial capital cost of the anaerobic processing system (Bitton et al., 2005).
7. The running cost of an anaerobic system is usually not energy intensive (Bitton et al., 2005).
8. The start - up phase of the process can be reduced by using acclimatized in the anaerobic conditions biomass, obtained by an existing corresponding and in operation unit (Lettinga 1995).

However, during this process some drawbacks are as well encountered:

1. The slow growth rate of the methanogenic archaea (Bitton et al., 2005).
2. Methanogenic archaea are sensitive to many toxic compounds (Lettinga 1995).
3. A long time for the acclimatization of the microbial population is required (Lettinga 1995).
4. The final output of the system usually needs further processing for safe and within limits disposal (Seghezzo et al., 1998).

3.2 Microbiology and metabolic pathways of anaerobic digestion

Biogas formation is also termed as biomethanation. Biologically derived gases are produced through a complex series of reactions. The process of anaerobic digestion is a result of combination of steps, in which the starting material is gradually broken down into smaller compounds. Specific groups of microorganisms are involved in each step. These organisms consecutively decompose the products of the previous step. The sequence of the entire organic matter decomposition lacks the necessity of adding an external electron acceptor.

Anaerobic digestion process occurs in four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Angelidaki et al., 2003). Figure 5 gives the schematic representation of the process and each step is described in detail below.

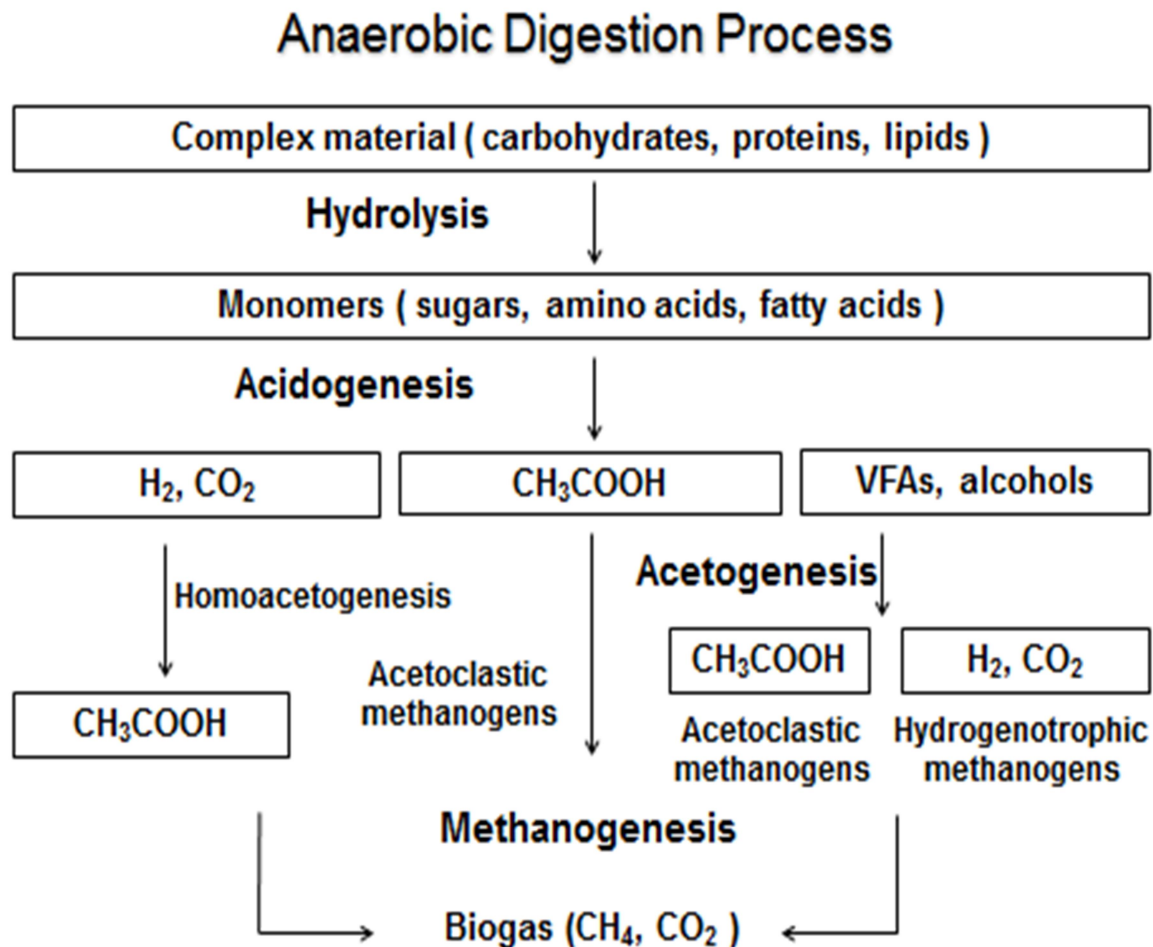
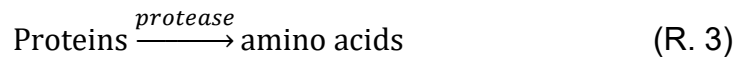
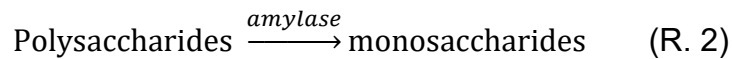
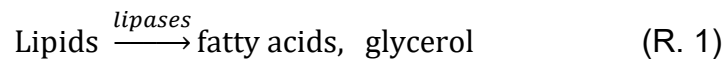


Figure 5: Schematic representation of the anaerobic digestion process.

3.2.1 Hydrolysis

In many cases, bacteria are not capable of directly absorbing the complex soluble or insoluble organic matter of the waste. Hence, these bacteria excrete enzymes that are capable of cleaving the large organic molecules into smaller soluble compounds. This extracellular process is often referred to as hydrolysis. Hydrolysis is the first step of anaerobic digestion. The complex organic material (polymer compounds) cannot penetrate the cell membrane of microorganisms. Thus, its decomposition into smaller compounds (mono- and oligomers) is mediated by extracellular enzymes that can penetrate the cell membrane. Carbohydrates, lipids and proteins are converted by hydrolytic enzymes in simpler and soluble compounds as shown in the following reactions.



According to Batstone et al., (2002), hydrolysis can be represented by two conceptual models:

- The organisms secrete enzymes to the bulk liquid where they adsorb onto a particle or react with a soluble substrate (Jain et al., 1992).
- The organisms attach to a particle, produce enzymes in the vicinity of the particle and benefit from soluble products released by the enzymatic reaction (Vavilin et al., 1996).

Hydrolysis is a relatively slow process and is regarded as the limiting step that will determine the overall rate of the process (Pavlostathis & Giraldo – Gomez, 1991). The parallel enzymatic steps account for the difference in hydrolysis rate of specific substrates. For the purposes of process design, hydrolysis determines the required retention time in a stirred methanogenic bioreactor (Angelidaki et al., 2011).

3.2.2 Acidogenesis

During acidogenesis the products of hydrolysis (sugars, amino acids and fatty acids) are microbially converted into more simple compounds such as low molecular weight organic acids, alcohols, carbon dioxide and hydrogen. Organic acids of low molecular weight include volatile fatty acids (acetate, propionate, butyrate, iso – butyrate, valerate).

This acid production process from sugars occurs without additional electron acceptor and is called fermentation. Microorganisms responsible for this process are mainly Clostridia and other Gram - positive bacteria (Ramsay et al., 2001).

The fermentation of the amino acids can occur in two ways:

1. via coupled oxidation reaction – reduction, Stickland reaction (Winter et al., 1987)
2. via oxidation of an amino acid with H^+ or CO_2 as the external electron acceptor.

The Stickland reaction occurs more rapidly and has specific features:

- an amino acid acts as an electron donor and it gets oxidized,
- a second amino acid acts as an electron acceptor and it gets reduced,
- the electron donor loses one carbon to CO_2 and a carboxylic acid is formed by one carbon atom less than the initial amino acid,
- the electron acceptor forms a carboxylic acid having the same carbon chain of the initial amino acid (Batstone et al., 2002).

Lipids are a good substrate for biogas production because of the high performance capability of methane (methane yield potential). Initially they are hydrolysed into glycerol and long chain volatile acids (LCFA). The latter are further converted through syntrophic acetogenic bacteria into hydrogen and acetic acid and finally to methane by the action of methanogenic archaea. Their degradation occurs through β - oxidation and it is referred as the limiting step of the process rate of anaerobic digestion. The LCFA inhibit the action of methanogenic microorganisms. This inhibitory effect was initially attributed to the toxicity resulting from cell destruction, affecting both syntrophic bacteria and methanogenic archaea. The absorption of the acids on the cell's surface was considered as the blocking mechanism, affecting the transfer of nutrients to the cell interior (Pereira et al., 2005). However, further studies showed that the inhibitory effect of LCFA are reversible and that after an adjustment phase the microorganisms are able to degrade LCFA sufficiently (Pereira et al., 2004). Usually the degradation is achieved via acidogenic bacteria which require interaction with hydrogenotrophic and acetotrophic methanogens through syntrophy (Sousa et al. 2007a, 2007b, Palatsi et al. 2010).

3.2.3 Acetogenesis

Acetogenesis is referred to the formation of acetic acid (CH_3COOH). Its formation is achieved by CO_2 reduction (homoacetogenesis) and mainly by the decomposition of organic acids. Acetogens using hydrogen for CO_2 reduction are called homoacetogens. They are strictly anaerobic bacteria and they use acetyl coenzyme A (acetyl - CoA). These bacteria compete with methanogens for substrates such as H_2 , formic acid and methanol (Angelidaki et al., 2011). Organic acids and alcohols

produced during acidogenesis are oxidized to acetic acid by the action of hydrogen producing acetogenic bacteria. Hydrogen ions (H^+) are used as electron acceptor. Under high hydrogen partial pressure ($> 10^{-4}$ atm), acetogenic reactions are not thermodynamically feasible, resulting in accumulation of metabolic intermediate compounds (acidogenesis products) and process failure.

In order to maintain the H_2 partial pressure at low levels, acetogens have to coexist with microorganisms able to consume it. Homoacetogens and hydrogenotrophic methanogens is an example. This coexistence is termed as syntrophy and the almost simultaneous production and consumption of hydrogen through syntrophy is called interspecies hydrogen transfer (Table 2). The symbiotic coexistence of acetogens and hydrogenotrophic methanogens is the only way to metabolise difficult degradable volatile fatty acids such as propionic acid.

Table 2: Standard Gibbs free energy of certain volatile fatty acids metabolic reactions through syntrophy.

Reaction	$\Delta G_o'$ (kJ/reaction)
<i>Oxidation reaction of volatile fatty acids</i>	
$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+ 76
$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$	+ 48
<i>Utilization of H_2 from methanogenic archaea and acetogenic microorganisms</i>	
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	- 136
$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$	- 105
<i>Propionic acid oxidation via syntrophy in conjunction with hydrogenotrophic methanogenesis</i>	
$CH_3CH_2COO^- + H_2 \rightarrow CH_4 + CH_3COO^-$	- 60

As shown in Figure 6, there is a thermodynamic “window” with partial H_2 pressure between 10^{-6} - 10^{-4} atm where conversions to acetic acid and methane are feasible.

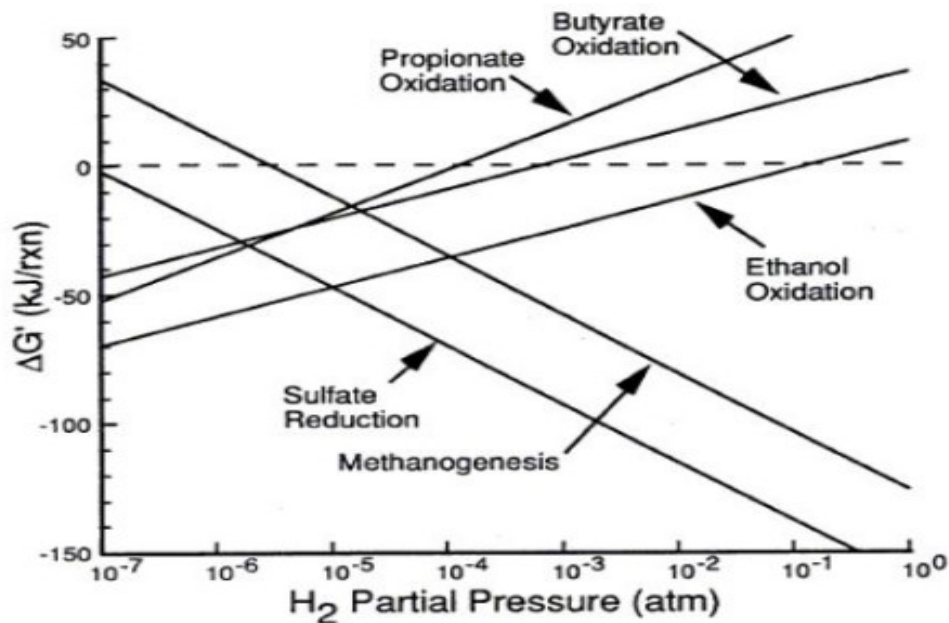


Figure 6: Effect of H_2 partial pressure on reactions involved in interspecies H_2 transfer during oxidation of ethanol, propionate and butyrate coupled to methanogenesis (Zinder 1993).

H_2 partial pressure has an effect and determines the stability of this process. For butyrate and propionate oxidations, coupled to methanogenesis, the thermodynamic “window” is narrow. Out of these values accumulation of volatile fatty acids occurs with possible process failure. Propionate oxidation is considered to be very easily perturbed in anaerobic bioreactors (McCarty, 1964). However, it has been suggested that physical juxtaposition between hydrogen consumers and producers facilitates hydrogen transfer (Conrad et al., 1985, Thiele et al., 1988).

3.2.4 Methanogenesis

Methanogens belong to the domain Archaea. There are five phylogenetic orders: *Methanomicrobiales*, *Methanobacteriales*, *Methanosarcinales*, *Methanococcales* and *Methanopyrales*. Methanogenic archaea are responsible for the final step of anaerobic digestion, the formation of methane (Angelidaki et al., 2011). Methane formation occurs from acetic acid as well as from CO_2 and H_2 . It can also be achieved by the conversion of formic acid or alcohols (Thauer et al., 2008). These microorganisms are strictly anaerobic.

Carbon source, pH value and temperature are parameters that are different for the optimal growth of the various methanogenic microorganisms. Some methanogenic archaea (*Methanobacteriales*, *Methanopyrales*) are exclusively using a specific metabolic pathway in terms of the methanogenic substrate, while other microorganisms can utilize alternative pathways (*Methanosarcinales*, *Methanomicrobiales*).

Methane formation can be achieved through the action of three main metabolic pathways (Conrad et al., 2010):

1. Acetotrophic methanogenesis
2. Hydrogenotrophic methanogenesis
3. Methylotrophic methanogenesis.

In the first case, acetic acid is decomposed into methane and carbon dioxide. In the second case, CO_2 is reduced to CH_4 . In the third case, methylated compounds (such as methanol, methylamines, methyl mercaptans) are converted to CH_4 .

Literature reports claim that acetate is the most important source of methane in anaerobic environments (Angelidaki et al., 2011) indicating that acetotrophic methanogenesis covers 70% while the remaining 30% concerns hydrogenotrophic methanogenesis (Conrad et al., 2010). The methylotrophic pathway is limited.

The main representative of acetotrophic methanogenesis is considered to be the order *Methanosarcinales*, while for hydrogenotrophic methanogenesis is the order *Methanomicrobiales* and *Methanobacteriales* (Bonin & Boone 2006, Garcia et al., 2006, Kendal & Boone 2006). For some microorganisms it is not clear which metabolic pathway are using since they have the ability to use both. Hydrogenotrophic methane production is part of syntrophy. Through interspecies hydrogen transfer the non-thermodynamically favoured acetogenesis is coupled with the thermodynamically favoured methanogenesis reaction.

3.3 Factors affecting anaerobic digestion process

The control of several parameters is required in order to ensure optimum operating conditions for an anaerobic system. Process efficiency and production rate of biogas are determined by specific factors. The role of each one of these parameters in the anaerobic digestion process is discussed in the following paragraphs:

Chemical composition and characteristics of the substrate: The chemical composition of the treated waste constitutes a factor of significant importance for the performance of an anaerobic system. The microbial community inside a digester is developed in accordance with the characteristics of the substrate. Those microorganisms that can easily metabolize the feeding components become dominant. Nutrients such as nitrogen and phosphorus in substrate should be in sufficient amounts otherwise addition of these nutrients is required. Also for optimum growth, methanogenic archaea require trace elements such as iron (Fe), molybdenum (Mo), cobalt (Co), nickel (Ni), and others. If they are not included in the chemical composition of the waste they can be added in the form of salts.

The amount of biogas to be produced and the content of methane both depend on the substrate, its biodegradability and oxidation state. Easily degradable material and the low oxidation state favour methane production (Angelidaki et al., 2011).

The existence, the number as well as the type and species of microorganisms under anaerobic conditions is dependent on qualitative and quantitative characteristics of the substrate (Hobson et al., 1974).

Temperature: It is one of the most important operating conditions of anaerobic digestion process. Its role is crucial to the growth of microorganisms and consequently to the produced biogas yield. Temperature affects the rate of biological reactions and bacterial growth.

Four temperature ranges characterizing microorganisms as to their optimal growth are classified:

- a) psychrothropic, $T < 20^{\circ}\text{C}$
- b) mesophilic, $T = 20 \text{ to } 40 - 45^{\circ}\text{C}$

c) thermophilic, $T = 45$ to 80°C

d) hyperthermophilic, $T > 80^{\circ}\text{C}$.

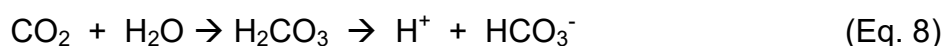
Methanogenic archaea are more sensitive compared to acidogenic bacteria in relation to temperature. This is also indicated by the low specific growth rate and the adjustment time required by methanogens upon temperature changes. A sudden change in temperature can be critical to the whole process.

The mesophilic and thermophilic conditions are indicated as the optimum conditions, with the second to outweigh the former. Thermophilic anaerobic digestion results in a higher rate of degradation and thus increased biogas efficiency. However, its high energy demand can automatically make it uneconomic thus giving mesophilic process an advantage as more easily implemented (Ward et al., 2008).

Alkalinity and pH: Anaerobic digestion is a process greatly dependent on pH. All microorganisms involved in this process exhibit optimum activity in a certain pH range. Methanogenic archaea and acidogenic bacteria show optimum growth at pH values close to 7 while acetogenic bacteria at pH values near 6. Methanogenic microorganisms grow slowly to a pH below 6.6 (Angelidaki et al., 2003).

Accumulation of volatile fatty acids during acidogenesis combined with inhibition of methanogenesis may result in suspension of the process. The continuous acid production can lead to acidity problems. Once pH is reduced, methanogenic activity is inhibited hence methane production as well. The system is possible then to reach the point of process failure. The high ammonia concentration is another factor likely to alter the pH. That can also result in instability issues of an anaerobic digestion system.

Alkalinity concerns the buffering capacity of a substrate entering the reactor. Organic matter degradation leads to carbon dioxide production which involves production of HCO_3^- . The equation (Equation 8) is the following:



High alkalinity value is in a position to allow safe operation of the system during possible variations in the pH. Otherwise, the system is not able to easily cope with sudden changes in pH with the risk of process inhibition.

Hydraulic retention time and organic loading rate: Hydraulic retention time (HRT) is the time for which the substrate and consequently the microorganisms remain in the reactor before exit the system. Is given by Equation 9:

$$\text{HRT} = V_R / Q_w \quad (\text{Eq. 9})$$

Where: V_R , reactor's volume (L) and Q_w , waste supply (L d^{-1} , daily flow rate). The hydraulic retention time is a critical design and operational parameter.

Initially, it is considered for calculating the volume of the digester. HRT corresponds to the average length of time a liquid is in a reactor. Is the length of time microorganisms have at their disposal in order to complete their metabolic functions, work on the substrate and produce biogas. Therefore, it becomes clear that hydraulic retention time must be sufficient for the organic matter degradation.

But beyond the time available to the microorganisms to degrade the organic content, the rate of the organic load holds an important role as well. The organic loading rate (OLR) of a system ensures the proper microorganism's activity.

Phenomena as an overload or an underload may lead to accumulation of acids and other inhibitory effects resulting in either complete failure of the process or a much reduced performance, respectively (Chynoweth et al., 1994, Marchaim & Krause 1993).

Toxic and inhibitory substances: Various compounds can have an inhibitory effect on the anaerobic digestion process. The presence of a toxic substance can result in a gradual decline of the performance. The resilience and adaptability of microorganisms determines the final outcome of the process in this case.

Methanogens are considered to be most sensitive in toxicity effects. However, the process can acclimatize, and higher concentrations of the toxicant can be tolerated after a period of adaptation (Angelidaki et al., 2003).

Two of the most common inhibitory factors for the anaerobic process are described below:

Oxygen: Strictly anaerobic microorganisms require complete absence of oxygen. However, there have been reported microorganisms resistant to oxygen's presence. These microorganisms listed as optional anaerobes.

Ammonia: Although ammonia is one of the necessary nutrients required for growth of microorganisms, it is possible to inhibit methane production when present in high concentrations. Therefore, ammonia is considered to be an inhibitory agent particularly in the anaerobic treatment of complex substrates such as animal waste (Yenigun et al., 2013). The ammonia is derived from the degradation of proteins. In waste as poultry, pig manure and slaughterhouse waste ammonia is found to inhibitory levels of concentration. The concentration of non - ionized form of ammonia (free ammonia, NH_3) has been identified as the responsible active ingredient for the process inhibition (Braun et al., 1981). The concentration of free ammonia depends on three parameters:

- total concentration of ammoniacal nitrogen
- temperature
- pH

Figure 7 illustrates the influence of these parameters on NH_3 dissociation.

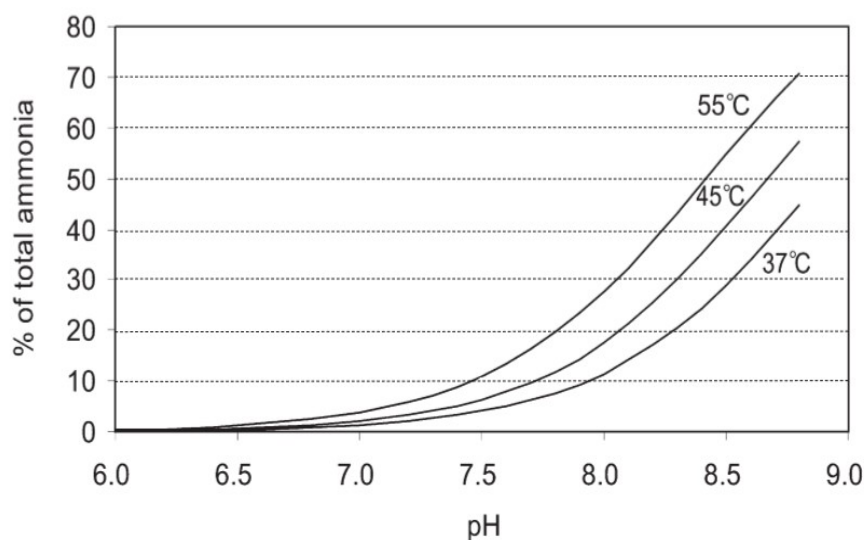


Figure 7: pH and temperature influence on NH_3 dissociation (Angelidaki et al., 2003).

With increasing temperature, the concentration of free ammonia increases. Generally, while the increase of temperature favors the metabolic growth of microorganisms at the same time leads to increased concentrations of the particular inhibitory agent (Braun et al., 1981, Hansen et al., 1998). Furthermore, pH value can determine the degree of ammonia ionization (Angelidaki et al., 2003).

Inhibition of anaerobic digestion due to increased concentration of free ammonia can result in volatile fatty acids accumulation. However, this leads to pH decrease, hence, free ammonia concentration is reduced to some extent. This interaction has been demonstrated by researchers as a sort of stabilization - mechanism when a balance between concentration of VFA and ammonia can be achieved (Angelidaki & Ahring 1994, Angelidaki et al., 2003).

Finally, according to Braun et al., 1981, an adaption period to ammonia can occur. However, if the possibility of adaptation of the microbial population is not feasible, then pH adjustment and appropriate choice of temperature is an easy and simple way to ensure a stable anaerobic process.

3.4 Biogas upgrading methods

During the anaerobic digestion process organic wastes are degraded and simultaneously energy is produced in the form of biogas. Biogas mainly contains CH_4 and CO_2 at percentages ranging between 40 – 75% and 25 – 60%, respectively (Luo & Angelidaki, 2012). Depending on the proposed end product of a biogas plant, cleaning and upgrading steps are required. The simplest case is when biogas is to be used directly. Raw biogas is used for electricity production, in combined heat and power (CHP) units, or directly for heat production. The need for biogas purification and the possible applicable processes in the case of biogas plants, with biogas upgrading, differ from those of on-site electricity conversion installations involving the direct use of raw biogas in CHP installations (Beil et al., 2013).

Biogas upgrading to CH_4 content higher than 90% can increase the heating value while extending its utilization possibilities as a renewable energy source (Deng & Hägg, 2010). Upgraded biogas (biomethane) can be used as vehicle fuel, or it can be injected into the existing natural gas grid transporting biomethane from rural

areas, where typically biogas plants are located, to urban areas where consumer density is higher (Luo & Angelidaki, 2012). This widens up the opportunities in distant energy consumption locations.

When biogas upgrading is performed, there are three major tasks involved: humidity removal, biogas desulphurization and CO₂ removal.

3.4.1 Humidity removal

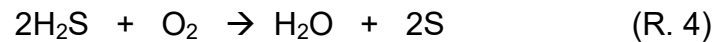
Biogas leaves the anaerobic reactor at a saturated with water vapour condition. The water condensates in gas pipelines and together with sulphur oxides it may cause corrosion (Petersson & Wellinger, 2009). This water must be removed from the gas flow in order for faults and disruptions in operation to be avoided during the following upgrading steps. The decrease of temperature in order for the water to condense from the gas flow constitutes an option. In practical application, biogas can pass through pipes installed in the soil and equipped with either a humidity trap or an electric cooler. Higher dew points can be reached through compression of the gas prior to the cooling step.

Water can also be separated from biogas with the use of silica gel (SiO₂), activated charcoal or molecular sieves. Absorption takes place. Under heating and/or pressure decrease the regeneration of these absorbing materials is possible. Other technologies for water removal are absorption in glycol solutions or the use of hygroscopic salts (Petersson & Wellinger, 2009).

3.4.2 Desulphurization of biogas

Desulphurization concerns the removal of hydrogen sulphide (H₂S). Raw biogas may contain H₂S in concentrations of < 100 mg / m_n³ up to 10,000 mg / m_n³ depending on the composition of the fresh substrate (Persson et al., 2006). H₂S removal is required in order to avoid corrosion effects in plant components. Two steps of desulphurization can be differentiated – primary and precision desulphurization (Beil & Beyrich, 2013). Primary desulphurization involves dosing of air or pure oxygen into the gas space of the anaerobic digester. This leads to biological oxidation of H₂S. By injecting a small amount of air (2–8% v/v) into the reactor's headspace, hydrogen

sulfide content can be biologically oxidized (Angelidaki et al., 2003) according to following reactions (R. 4, R. 5):



The reaction occurs spontaneously and free sulphur or aqueous sulphurous acid is generated.

It is considered to be a simple and economical technique of reducing H_2S level to < 500 ppm (Beil & Beyrich, 2013). The use of air includes the presence of inert nitrogen (N_2). The N_2 accumulation in the raw gas and its subsequent dilution is a disadvantage which does not occur with the use of pure oxygen.

Precision desulphurization is the method which decreases hydrogen sulphide concentration at really low level, less than 5 mg / m_n^3 (Beil et al., 2013). Activated carbon is used for catalytic oxidation and adsorption. For the catalytic H_2S oxidation a small amount of oxygen is needed. This can be provided by the primary dosing of air or oxygen into the digester. Alternatively, chemical precipitation using iron salts (sulphide precipitation) or chemical precipitation using iron hydroxide in an external column can be applied for precision desulphurization (Beil & Beyrich, 2013).

3.4.3 CO_2 removal

Regarding CO_2 removal, the upgrading technologies can be assigned to the following groups:

- absorption
- adsorption
- permeation
- cryogenic upgrading.

Absorption technology including water scrubbing, organic physical scrubbing and chemical scrubbing together with the technology of pressure swing adsorption (PSA), high-pressure membrane separation and cryogenic upgrading are the most widespread technologies. Key aspects of each technology are briefly discussed in turn.

3.4.3.1 Absorption technology

Through this process carbon dioxide as well as hydrogen sulphide can be removed from biogas. The different binding forces of the more polar CO₂ or H₂S and the non-polar methane are used to separate these compounds (Persson et al., 2006). Absorption implies the dissolution of gas or vapour in a liquid (Beil & Beyrich, 2013). Raw biogas combined with a liquid flow is inserted into a column filled with plastic packing material. Thus, the contact area between both the gas and the liquid phase is increased. In principal, carbon dioxide is more soluble than CH₄. Hence, the liquid outflow of the column will contain increased concentration of carbon dioxide, while the gas leaving the column will have an increased concentration of methane (Petersson & Wellinger, 2009).

Water scrubbing: When water is used as solvent then the process is called water scrubbing. Raw biogas is compressed and fed into a column. It is added from the bottom of this column where it meets a counter flow of water (Persson et al., 2006). The column is filled with packing material so as a large contact surface between the gas and the liquid to be created. Higher solubility of CO₂ results in its dissolution to a higher extent in water than methane, especially at lower temperatures. Inside the scrubber column carbon dioxide is dissolved, while in the gas phase the methane concentration is increased. Therefore, the biogas which is brought out of the top of the column is enriched in methane and saturated with water (Persson et al., 2006). A drying step is required for the reduction of the water vapour. The CO₂ – enriched water, leaving the absorption column, is transferred to a flash tank. The dissolved gas is released and transferred to the raw gas inlet. In the case of water recirculation, it is transferred to a desorption column. There carbon dioxide meets a counter flow of air, into which it is released. The water is cooled down to achieve the large difference in solubility between methane and carbon dioxide before it is recycled back to the absorption column (Petersson & Wellinger, 2009).

Organic physical scrubbing: This process involves also physical absorption. However, in contrast with water scrubbing, an organic reagent such as polyethylene glycol serves as absorption agent (Beil & Beyrich, 2013). The process follows the principle of water scrubbing. The main difference is that carbon dioxide and hydrogen sulphide are far more soluble in organic solvents than in water (Persson et

al., 2006). Hence, the same gas capacity corresponds to a smaller biogas upgrading unit. Compared to water scrubbing, the increased absorption rates of the organic reagent result in reduced recirculation rates of the absorption liquid. Regeneration can occur through heating or depressurizing. Prior to the insertion of the untreated gas to the absorption column, compression is required. Cooling of the compressed gas causes condensation of water, which can be diverted from the system (Beil & Beyrich, 2013). Examples of organic solvents used in organic physical scrubbing are the trade names of Selexol® and Genosorb®.

Chemical scrubbing: Chemical absorption with organic solvents is usually termed as amine scrubbing. Amine solutions are used. For biogas upgrading the alkanol amine solutions used are mono ethanol amine (MEA) and di-methyl ethanol amine (DMEA) (Petersson & Wellinger, 2009). CO₂ is not only absorbed in the organic solution, but in addition, it reacts chemically with the amine. Due to evaporation, part of the liquid is lost and its replacement is needed. Carbon dioxide is absorbed during the amine scrubbing process and the liquid in which is chemically bound gets regenerated by heating. When hydrogen sulphide is present in the raw biogas, it is co - absorbed. In that case, higher temperatures are required for the regeneration. Therefore it is advisable to remove it before absorption in the amine scrubber (Petersson & Wellinger, 2009). In most applications, a precision desulphurization step is installed before the absorption column to reduce the energy demand for the regeneration process (Beil & Beyrich, 2013). The end product gas is saturated with moisture, and must therefore be dried in order to protect downstream equipment. Because of very low methane losses in the system, the off-gas does not normally require further treatment (Beil & Beyrich, 2013). Finally, either its distribution into the gas grid or its use as a vehicle fuel can occur.

3.4.3.2 Pressure swing adsorption (PSA)

This process constitutes an adsorptive biogas upgrading technology. Adsorption occurs through the deposition of constituent parts of gas, in this case CO₂, onto the surface of solid matter (Beil et al., 2013). The solid matter is referred to as adsorbent. Zeolites, activated carbon or molecular sieves are mainly used. This method takes place under elevated pressure. The adsorbing material is regenerated by reducing the pressure and by a subsequent application of a light vacuum

(Persson et al., 2006). In practical application, a PSA upgrading unit has several vessels working in parallel. Once the adsorbing material in one vessel becomes saturated, the raw gas flow is switched to another vessel in which the adsorbing material has been regenerated (Petersson & Wellinger, 2009). The regeneration is completed through stepwise depressurization. The desorbed gas, during the pressure drop, it may contain methane that was adsorbed along with carbon dioxide. Hence, depending on the methane quantity, the desorbed gas can be either led to the following column or it may be released if it is methane free. Apart from CO₂, other constituent parts of gas can also be retained, such as water (H₂O) or hydrogen sulphide (H₂S) (Beil et al., 2013). If raw biogas containing H₂S is applied, the latter will be irreversibly adsorbed. In addition, PSA method requires no humidity. Water can destroy the structure of the adsorbing material. Therefore, the aforementioned components need to be removed before the PSA-column.

3.4.3.3 High – pressure membrane separation

Membrane separation, also known as gas permeation, takes advantage of the different permeabilities of gas compounds through polymer membranes (Beil et al., 2013). Cellulose acetate or aromatic polyimides are examples of the used polymers. Molecules of different size demonstrate different permeability through the membrane. Three different flows can be defined in membrane systems: the feed (raw biogas), the CO₂-rich permeate and the CH₄-rich retentate (Beil & Beyrich, 2013). By providing a driving force for separation, in the form of partial pressure difference between the two sides of the membrane, quantitative separation of gas components is performed (Miltner et al., 2016). Usually membranes are in the form of hollow fibres bundled together (Petersson & Wellinger, 2009). During this process high permeability levels are exhibited for CO₂ and H₂S whereas methane is retained. In order to extend the turnaround time of the membranes and to guarantee an optimum performance, apart from a drying and fine desulphurisation step of raw biogas, the latter passes through a filter that retains water and oil droplets and aerosols, which would otherwise negatively affect the membrane performance (Petersson & Wellinger, 2009, Beil & Beyrich, 2013). In practical applications, the process occurs in at least two stages. The permeate stream is not methane free, hence the exhaust gas is either recirculated or it is led to an additional stage.

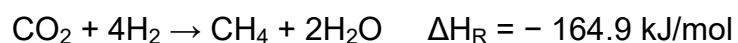
3.4.3.4 Cryogenic upgrading

Cryogenic upgrading process employs the distinct boiling/sublimation points of the different gases, particularly for the separation of carbon dioxide and methane (Petersson & Wellinger, 2009). Methane has a boiling point of -160°C at atmospheric pressure whereas carbon dioxide has a boiling point of -78°C (Persson et al., 2006). As a result, CO_2 can be separated from biogas in a liquid form by implementing a cooling step at elevated pressure. The principle of cryogenic separation is that biogas is compressed and then cooled by heat exchangers followed by an expansion step for instance in an expansion turbine (Persson et al., 2006). Through the cooling and the expansion steps, CO_2 is forced to condensate, while CH_4 accumulation in the gas phase occurs. After the removal of CO_2 as a liquid, a further cooling step for the gas takes place to condensate methane.

Generally, all the aforementioned upgrading methods are performed outside the anaerobic reactor and require investments in external equipment (Luo & Angelidaki, 2013). Therefore, the cost is relatively high. The main disadvantage is that small amounts of CH_4 are also removed together with CO_2 , thus creating increased greenhouse gas emissions (Weiland P. 2010, Nordberg et al., 2012). A concept that can actively contribute to the biogas upgrading process is the Power to Gas or better yet Power to methane system.

3.5 Power to Gas concept – Hydrogen utilization in anaerobic digestion process

Power to Gas system involves renewable electricity conversion to hydrogen (H_2). Electrolysis can be employed when electricity overproduction is combined with low demand. Hydrogen utilization in the anaerobic digestion process can then take place. Hydrogen can be used for methane (CH_4) production through methanation. There are two ways: i) catalytic methanation and ii) biological methanation. Both proceed via the Sabatier reaction (Reaction 6) (Tarancón et al., 2016).



$$\Delta G_{r298k} = 130.8 \text{ kJ/mol} \quad (\text{R. 6})$$

Since the process results in methane production, the concept is more accurately referred to as Power to methane. Nonetheless, for the potential of power to methane concept the source of carbon dioxide (CO_2) is also important.

The CO_2 can either be of fossil or renewable origin, extracted from the air or various industrial waste gases (Persson et al., 2014). CO_2 can be captured as an exhaust gas from a thermal power facility, therefore allowing PtG to be considered as a form of carbon capture. However, CO_2 capture can be expensive. Estimates for the cost present coal plants as the lower end of a wide price range and the combined gas turbine plants as the upper end (IPCC 2005, Sterner 2010). Alternatively, other processes can result in CO_2 production in a more economical way. Biogas plants are an example where CO_2 comes from biogas upgrading free from contaminants in most cases (Ahern et al., 2015).

Hence, the power to methane concept can have a great potential at a biogas plant. There are different ways that renewable hydrogen can be integrated into a power to methane concept. A depiction of the possible pathways is given in Figure 8.

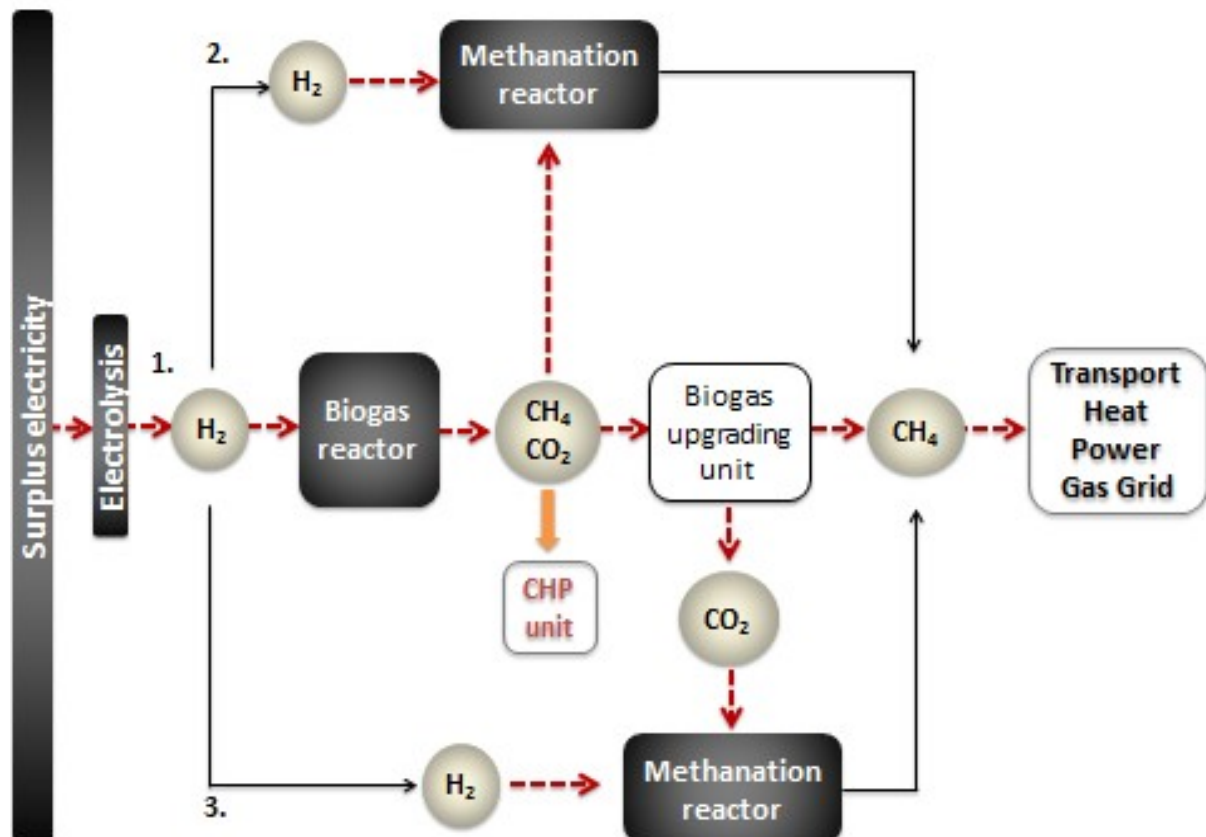


Figure 8: Different pathways of power to methane concept at a biogas plant.

The first pathway (1) involves *in – situ* hydrogen addition into the anaerobic reactor. An additional upgrading step is also included. The other options both are depicting the *ex – situ* use of hydrogen. Methanation process is taking place in a separate reactor. It can occur in a catalytic or a biological way. The difference between pathway 2 and 3 is that as CO₂ source, biogas and CO₂ coming from the upgrading unit is used, respectively.

Overall, surplus electricity can be converted into an easily storable gas such as methane and the existing infrastructure system can facilitate the storing of electricity that otherwise would be curtailed.

The different ways that hydrogen can be combined with CO₂ for methane production are described below.

3.5.1 Catalytic methanation

Extensive studies have been conducted on catalytic removal of CO₂. This process can be described by Sabatier reaction (Reaction 6) that was introduced in Section 3.5. The Sabatier equation is an exothermic reaction which proceeds catalytically. Nickel (Chang et al., 2003, Yamasaki et al., 2006), Ruthenium (Mori et al., 1996, Rynkowski et al., 2000) and Rhodium (Bowker et al., 1993) were used as metal-based systems. Bimetallic and bifunctional catalytic concepts were also investigated (Park & McFarland, 2009). Catalytic methanation is thermodynamically favoured at high pressure. It is an exothermic reaction generating significant quantities of heat. Temperature control is essential to favour methane formation and to avoid overheating and deterioration of the catalysts (Persson et al., 2014). According to Benjaminsson et al., (2013), a nickel or a ruthenium-based catalyst requires 300 to 500°C, giving an overall energy conversion efficiency of 80%.

Catalytic methanation can be carried out at a biogas plant site. The drawback, though, is that it has to be implemented after the biogas upgrading step or at least after desulphurization. Impurities of biogas (such as hydrogen sulfide) have to be removed. The clean concentrated stream of CO₂ participates in the catalytic step. The case for biological methanation is different.

3.5.2 Biological methanation

Biological methanation is a second option for Power to Gas process chain. In this case methanogenic microorganisms serve as biocatalysts (Götz et al., 2016) and Sabatier reaction (Reaction 6) can occur. Hydrogenotrophic methanogens can obtain energy through anaerobic metabolism of hydrogen and carbon dioxide. Biological methanation requires mild operational conditions such as temperature between 20 - 70° C and mainly ambient pressure.

Biological methanation can take place prior to the biogas upgrading step since raw biogas can react with hydrogen. There are two process concepts: *in – situ* and *ex – situ* methanation.

3.5.2.1 *In – situ* biological methanation

The process where hydrogen is added to the anaerobic reactor and reacts with raw biogas within the biogas digester is termed *in – situ* biological methanation (Persson et al., 2014).

Hydrogen is directly fed into the anaerobic reactor. CO₂ already exists in the produced biogas, it is produced by acetoclastic methanogens. However, CO₂ is inert as a fuel. It dilutes biogas energy content. Hence, a part of CO₂ can be *in – situ* converted to methane. This process results in biogas with higher methane content and a higher calorific value.

In theory, based on Sabatier reaction (Reaction 6) if hydrogen is inserted to the reactor at 4 times the quantity of the CO₂ then 100% efficiency in principle will occur. In practice, full conversion is rarely obtained. Hydrogen is much less soluble than CO₂. Gas – liquid mass transfer is a crucial parameter. It defines whether hydrogen will be available for consumption. The literature contains details of different types of reactors (Lee et al., 2012, Burkhardt et al., 2013), different ways of inserting hydrogen into the system (Luo & Angelidaki, 2013a) as well as different packing materials (Bassani et al., 2016) and the effect each of these factors has efficiency of the process. According to Persson et al., (2014) in this process it is unlikely that a biomethane standard (> 97% methane content) suitable for gas grid injection or for vehicle use will be achieved. Thus, a smaller biogas upgrading step will be required if biomethane is the proposed end product.

However, *in-situ* biological methanation with raw biogas can result in a significant decrease on the costs associated with biogas upgrading providing a sustainable financial option.

3.5.2.2 *Ex – situ* biological methanation

In *ex – situ* methanation process the conversion of hydrogen and carbon dioxide takes place in a separate reactor. Through this process the coupling of hydrogen with CO₂ does not necessarily have to interfere to an already existing anaerobic digestion plant. A completely separate reactor with much smaller volume can be implemented for high methane content (Luo & Angelidaki, 2012).

This concept offers different possibilities. Separating the processes into separate reaction vessels, allows the conditions to be optimised for each process. The used inoculum can be enriched with hydrogenotrophic methanogens. Guneratnama et al., 2017 investigated the use of mixed culture and enriched culture. The operational conditions can be adjusted exclusively to the requirements of hydrogenotrophic methanogens. Luo & Angelidaki (2012) investigated the effect of the mixing intensity on the efficiency of the process. Furthermore, the CO₂ used can be the clean concentrated stream after the biogas upgrading step or from another source.

Ex – situ biological methanation can either contribute to the decrease of biogas upgrading cost or to the use of the CO₂ originating from the upgrading step itself.

Overall, biological merhanation process presents some positive aspects. Hydrogen and carbon dioxide can be both employed into the anaerobic digestion process. Biological methanation can be a form of carbon dioxide capture. Hence, the aforementioned technologies of CO₂ removal can be either partly or completely bypassed. Also compared to catalytic methanation, it can be implemented before biogas upgrading step. Impurities of biogas do not necessarily have to be removed since no catalyst is needed.

It is important to evaluate the stability and the efficiency of both *in – situ* and *ex – situ* biological methanation processes since through a biological way, surplus electricity can be converted into an easily storable gas such as methane and the existing infrastructure system can facilitate the storing of electricity that otherwise would be curtailed.

4. Materials and methods – Experimental configuration

4.1 Wastewater characteristics and feedstock preparation

During this research work for both batch and continuous experiments sewage sludge was used as a substrate. Samples of sewage sludge were obtained from Kesslerfeld wastewater treatment plant (WWTP) in Duisburg, Germany. Substrate corresponded to a mixture of primary and secondary sludge. The mixture was gathered in a thickening tank used for sludge recirculation. The samples were taken after this sedimentation step. A part of this substrate was used immediately. The remaining amount was stored in -18°C . Such a low temperature could ensure that organic matter degradation will not occur until the samples were thawed and ready to be used (Zanoni 1965, Berg 1982, APHA 1989). In order to adjust the organic volatile solid content to the required operational conditions dilution with tap water took place.

Furthermore digested sludge was also obtained from the same wastewater treatment plant. It corresponded to the effluent of the anaerobic reactors of the plant. This sample was used as an inoculum for this experimental set-up.

For the characterisation of both the substrate and the inoculum the main parameters were determined. In Table 3 the average along with standard deviation values are given.

Table 3: Characterization of substrate and inoculum

Parameter	Value	
	<i>Substrate</i>	<i>Inoculum</i>
TS (g/L)	35.8 ± 1.8	20.5 ± 1.8
VS (g/L)	23.9 ± 1.2	11.3 ± 1.2
% VS/TS	67.5 ± 2.4	55.2 ± 2.3
TSS (g/L)	38.2 ± 0.7	18.2 ± 0.4
VSS (g/L)	31.4 ± 0.8	13.1 ± 0.3
total COD (g/L)	23.4 ± 1.5	10.9 ± 0.7
soluble COD (g/L)	15.9 ± 0.8	7.3 ± 0.4
$\text{NH}_4^+\text{-N}$ (mg/L)	1138 ± 263	1214 ± 117
pH	7.3 ± 0.2	6.7 ± 0.1

Throughout the whole experimental period the substrate was supplemented with a trace metal solution modified from Hussy et al., (2004). Detailed composition of the trace metal solution is given in Table 4.

Table 4: Modified composition of the trace metal solution (Hussy et al., 2004).

Compound	Concentration (mg L ⁻¹)
NH ₄ Cl	2600
K ₂ HPO ₄	250
Na ₂ MoO ₄ * 6H ₂ O	14
FeSO ₄ * 7H ₂ O	86
MgCl ₂ * 6H ₂ O	320
CaCl ₂ * 6H ₂ O	66
MnCl ₂ * 4H ₂ O	15
CoCl ₂ * 6H ₂ O	15
CuCl ₂ * 2H ₂ O	10
NiCl ₂ * 6H ₂ O	49
ZnCl ₂	23

This nutrient solution was added to the feeding substrate to ensure sufficient supply of trace metals, nitrogen and phosphorus. The solution was prepared in 10-fold concentration. After acidification with HCl to a pH value of ~ 2 the solution was stored at 4° C (Hussy et al., 2004).

4.2 Experimental configuration

The experimental set – up consisted of a feeding tank followed by the main reactor. Two continuously stirred tank reactors (CSTR) made of Plexiglas were constructed. Total volume of each reactor was 11.3 L. The working volume was 9.5 L. The first reactor (CSTR₁) was stirred by an adjusted mechanical stirrer. The second one (CSTR₂) via magnetic stirrer. Temperature control was accomplished by using a thermal bath (Julabo GmbH F12) with water recirculation through the reactors' double jacket. Input and output were inserted and removed respectively with the use of Heidolph (Schwabach, Germany) tube pumps. Set timers were controlling the

feeding intervals. The second reactor was slightly modified in order to achieve the injection of hydrogen gas into the system. In Figure 9 and Figure 10 a simplified configuration of the experimental set-up for both systems is mapped.

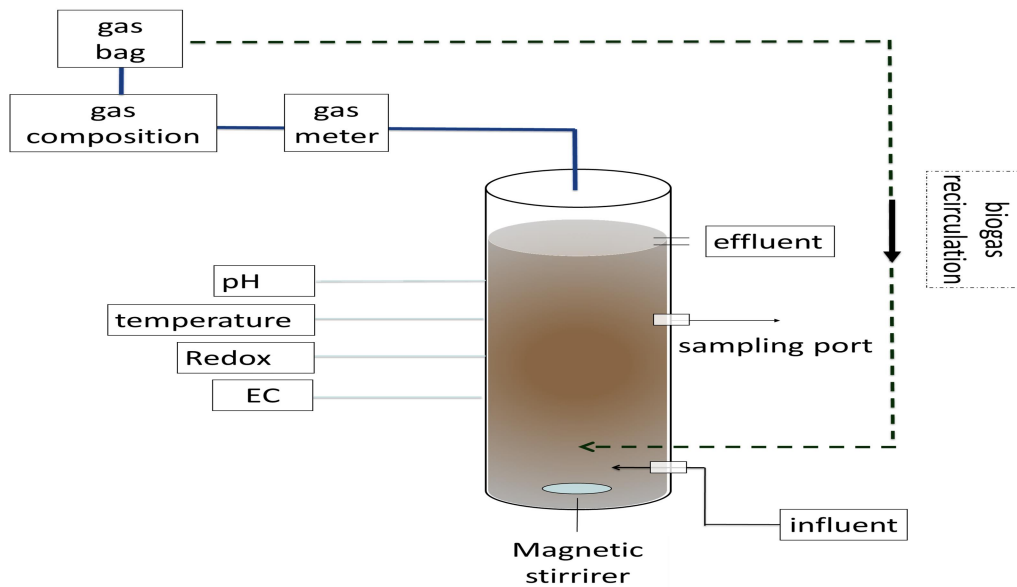


Figure 9: Schematic configuration of the 1st experimental system (CSTR₁).

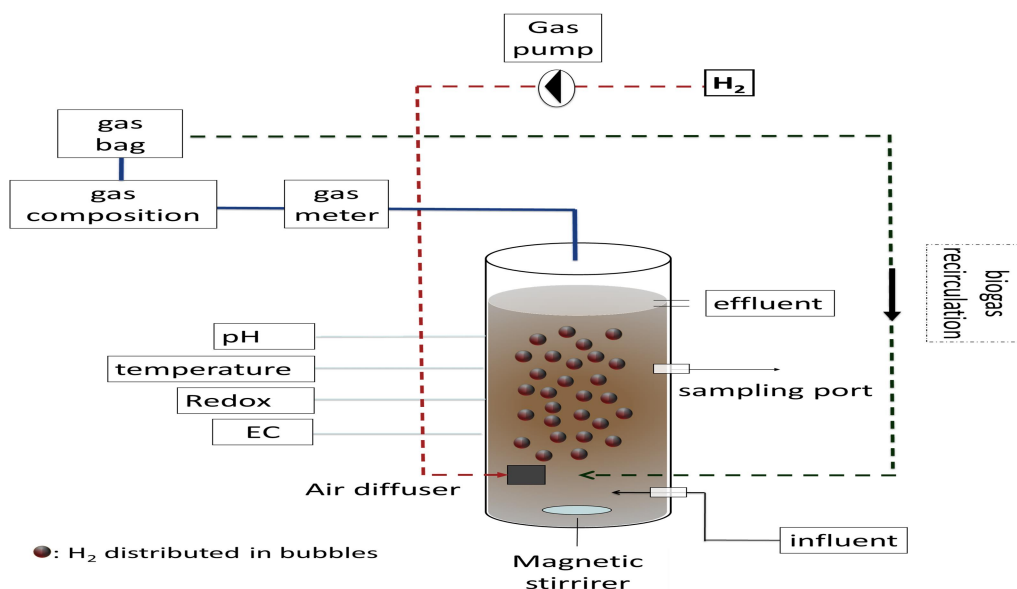


Figure 10: Schematic configuration of the 2nd experimental system (CSTR₂).

For monitoring the operation IKS – aquastar probes (IKS Computersysteme GmbH) were installed. Adjusted and calibrated according to the manufacturer's instructions, probes for pH, electrical conductivity (EC), Redox potential and temperature values were used. All experiments were conducted at a mesophilic temperature (37° C). Hydrogen entered the liquid phase of the reactor through ceramic gas diffusers. Biogas volume and composition were measured. Produced biogas was also collected in gas sampling bags (Restek GmbH) for further recirculation into the system.

4.3 Analytical methods

During this research work the chemical determination of various parameters was performed. The goal was through the analysis to obtain a complete picture of the progress of the anaerobic digestion process. Additionally, it was aimed to investigate the response of the system in scheduled or unscheduled disturbances and changes of the operating conditions.

Initially, waste and inoculum characterization was held as it was mentioned before. Chemical oxygen demand (COD), total and volatile solid concentration (TS and VS) was determined. Further analysis concerned the concentration of ammoniacal nitrogen (NH_4^+ - N). Finally, pH and electrical conductivity (EC) values were checked.

During the whole process representative samples were taken from designated points of the experimental configuration.

In addition to the above mentioned parameters, the complete analysis of the samples included the determination of the concentration volatile fatty acids (VFAs).

It should be noted that the determination of the concentration of the soluble fraction of chemical oxygen demand and the volatile fatty acids, related to a sample, was taken after vacuum filtration through a membrane, cellulose acetate filter (Sartorius AG) with a porosity of 0.45 μm or 0.2 μm (APHA 1989).

The samples were mostly analysed on the same day of sampling. Alternatively, the samples were frozen and analysed in the following days, in accordance to literature

reports (Zanoni 1965, Berg 1982, APHA 1989). The recording and measurement of biogas and content of methane was taking place daily.

The analytical methods of measuring these parameters are described in Section 4.3.1.

4.3.1 Total and volatile solid concentration

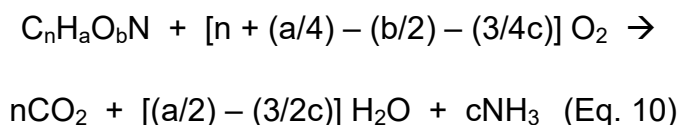
The determination of total and volatile solids was carried out based on the method described in the book «Standard Methods for the Examination of Water and Wastewater», (APHA, 1989). After evaporation at 103 - 105° C, the remaining fraction of a sample represents the concentration of total solids (TS). A known amount of the sample was placed in a pre-weighed porcelain crucible. The next step was evaporation in a drying oven until constant weight. The sample was then placed in a desiccator equipped with a desiccant containing a colour indicator of moisture concentration in order to balance temperature and weight. Additional placement at 550° C in a muffle furnace, leads to the identification of volatile solids (VS) concentration. Cooling, desiccating and weighting are the final steps before the calculations. Since the samples corresponded to a high solid content, measuring accurately specific volume proved to be difficult. Hence the solid concentration was examined both in g/L and g/kg. The % of solids was used as well for calculation reasons.

For the determination of total suspended solids (TSS) and volatile suspended solids (VSS), there was a difference in regard to the method. Suspended solids are defined as the non-filtered solids. For this reason the sample was filtered on a pre-weighed filter. More specifically, the filter was placed on a sieve. Then it was screwed under a filter vessel connected to a compressed air valve and the volume was filtered through. Finally, for the filters with the non-filtered solids content, the heating process as for total and volatile solids was followed.

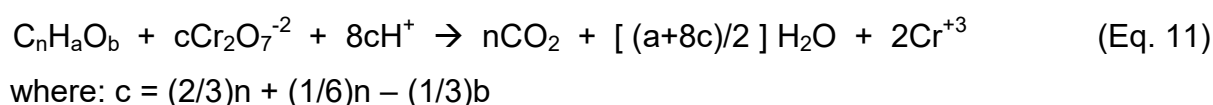
4.3.2 Chemical oxygen demand

The determination of Chemical Oxygen Demand (COD) took place according to Standard methods, APHA (1989). By measuring this parameter the organic strength of the desired solution is determined. COD is defined as the total amount of oxygen

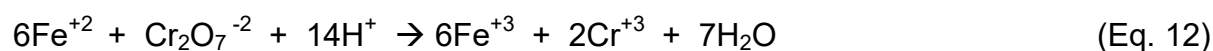
required for complete oxidation of carbon to carbon dioxide and water. The oxidation reaction takes place in accordance with Equation 10:



Most organic compounds, under the presence of a strong oxidant in acidic environment, can be oxidized. Based on this chemical property, oxidation of the organic content of a solution using an excess of potassium dichromate ($K_2Cr_2O_7$) can be obtained. Heating to $148^\circ C$ in strongly acidic conditions is required. A prerequisite is that the solution should not contain a chloride ion concentration (Cl^-) of more than 2 g Cl L^{-1} . For the oxidation of volatile aliphatic compounds silver sulphate ($AgSO_4$) is used as a catalyst. Halide ions are capable of reacting with $AgSO_4$ and form partially oxidized sediments. This can lead to error. To avoid this, prior to heating the samples, mercury (II) sulphate ($HgSO_4$) is added. Mercury ions lead to the formation of complexes with halide ions. The oxidation reaction (Equation 11) of the organic material from dichromate ions is described by the equation below:



Closed reflux titrimetric method was applied to the remaining chromium (IV) ions (Cr^{+6}). These cations, derived from unreacted $K_2Cr_2O_7$, were titrated with a hydrate complex solution of ammonium iron (II) sulphate hexahydrate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, FAS). The equation (Equation 12) taking place is as follows (Sawyer & McCarty, 1978):



In the present study both total and soluble chemical oxygen demand were measured. The soluble fraction resulted after filtration of the total fraction through membrane filters. Soluble chemical oxygen demand (sCOD) was also determined by Hach Lange cuvette tests and evaluated by a DR2800 Hach Lange Spectrophotometer (Hach Lange GmbH). The Chemical Oxygen Demand is expressed in mg COD L^{-1} .

4.3.3 Volatile fatty acids

The determination of volatile fatty acids (VFAs) was performed by using a gas chromatograph (Autosystem XL Gas Chromatograph, Perkin Elmer Instruments) equipped with a flame ionization detector (FID). The flame was obtained by hydrogen combustion with air supply. The carrier gas was helium (He) which was carrying forward the sample in the column with a flow of 2.6 mL min^{-1} .

The sample, after filtration with membrane filter of $0.2 \text{ }\mu\text{m}$, was ready for analysis. By using micro syringe, the sample was injected into the chromatograph. A temperature program with duration of about 17 minutes was used in column. The determination of the concentration of the acids was made by utilisation of 'standard curves'. These curves were a result of measurements made for standard acid solutions of very high purity, for various dilutions. n – butanol was used as an internal standard. The operating conditions of the gas chromatograph are summarized in Table 5.

Table 5: Operational conditions of gas chromatograph during the analytical determination of VFAs.

Column	Fused silica capillary FS-FFAP-CB
Column temperature	Initial: $90 \text{ }^{\circ}\text{C}$, constant for 2 min Increase with pace $20 \text{ }^{\circ}\text{C/min}$ until $220 \text{ }^{\circ}\text{C}$ constant at $220 \text{ }^{\circ}\text{C}$
Carrier gas	He (14 psi)
Carrier gas flow	2.6 mL/min
Detector	Flame ionization (FID)
Detector temperature	$260 \text{ }^{\circ}\text{C}$
Injector volume	$1 \text{ }\mu\text{L}$
Injector temperature	$240 \text{ }^{\circ}\text{C}$
Split	1 : 3.7
Duration of analysis	17 min

4.3.4 Ammonium and phosphorous concentration

Previously it is stated that the soluble fraction of chemical oxygen demand (sCOD) was determined by Hach Lange cuvette tests and evaluated by a DR2800 Hach Lange Spectrophotometer. Under the same process ammonium and phosphorus concentration were measured as well. After filtration through a 0.45 μm cellulose acetate filter (Sartorius AG), a specific cuvette test was used for each parameter as indicated in Table 6.

Table 6: Analytical methods for the determination of sCOD, NH_4^+ – N and PO_4^{3-} – P concentration.

Parameter	Cuvette test / Concentration range	Determination principle
sCOD	LCK 514 100-2000 mg / L O_2	Dichromate
	LCK 014 1000-10000 mg/L O_2	
NH_4^+ – N	LCK 305 1.0-12.0 mg/L NH_4 – N	Indophenol blue
	LCK 302 47-130 mg/L NH_4 – N	
PO_4^{3-} – P	LCK 348 0.5 – 5.0 mg/L PO_4 – P	Molybdenum blue

4.3.5 Biogas volume and composition

Gas volume was measured during the continuous experiments by a wet gas-meter supplied by Ritter Kunststoffwerk KWU B (Ritter Apparatebau GmbH & Co. KG, Germany). Biogas Composition (carbon dioxide (CO_2), methane (CH_4) and hydrogen sulfide (H_2S) content) was determined using a BIOGAS 5000 gas analyzer (Geotechnical Instruments LTD, UK).

For the first two main components the analyzer was equipped with dual wavelength infrared sensor with reference channel and for hydrogen sulfide with an internal electrochemical cell.

For monitoring a more complex composition of biogas, that included hydrogen, a stationary process gas analyzer based on modular system from TAD Gesellschaft für Elektronik-Systemtechnik mbH, Type GME.84.D-K4 was used. Calibration was carried out according to the manufacturer's instructions. CO_2 and CH_4 percentages were determined by a NDIR (Nondispersive Infrared) sensor and H_2 percentage by a

thermal conductivity sensor. Depending on the gas analyzers minimum volume requirements and the hazard of handling all these gas components, separately or as a mixed gas, sampling bags (Restek GmbH) were used.

4.3.6 Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) is a technique using fluorescently labeled probes which was first described in 1989 by DeLong. It has become a widely used method for examining the microbial consortia in a variety of environmental systems. The principle of this technique is the hybridization of fluorescently labeled probes to ribosomal rRNA in permeabilized whole microbial cells. Short pieces of DNA, 15–25 nucleotides in length, are consisted in these probes. They are designed to specifically hybridize to their complementary target sequence on the rRNA structures in the target cell. Depending on the composition, the probe can specifically target a narrow phylogenetic group or any other higher phylogenetic hierarchical group (Amann et al., 1995).

The applied protocol for FISH analysis in the present work is according to the book 'FISH Handbook for Biological Wastewater Treatment - Identification and quantification of microorganisms in activated sludge and biofilms by FISH' edited by Nielsen et al., 2009. The main steps of the protocol are briefly described below:

- Fixation of the sample: A part of ethanol 96% along with an equal volume of the sample are transferred in a 50 ml tube. 4 to 16 h of incubation is required. This step inactivates microbial cells and allows permeabilization of the cells for probe penetration.
- Sample application: Once slides are cleaned by ethanol, 10 μ L of sample is added to each well and the slide goes to the drying oven at a temperature of 46°C. Then solutions with increasing ethanol concentrations (50%, 80% and 96% for 5 min each) are used for the dehydration of the sample. The slides are simply dipped in these solutions. Drying step is now repeated.
- Hybridization buffer: In a 50 ml tube a hybridization buffer solution is prepared. The way of preparation must correspond to the formamide concentration empirically optimized for each applied gene probe (Details on oligonucleotide

probes are available at <http://www.probeBase.net>, Loy et al., 2003, Greuter et al., 2005, Loy et al., 2007). The selected formamide concentration should prevent the loss of signal intensity of the target cells. Table 7 includes the composition of hybridization buffer for each percentage of formamide used during the experimental work.

Table 7: Composition of the hybridization buffer at the applied formamide concentrations.

Formamide Concentration	20%	25%	35%	45%	50%
5 M NaCl (μL)	360	360	360	360	360
1 M Tris-HCl (μL)	40	40	40	40	40
Formamide (μL)	400	500	700	900	1000
Deionized H ₂ O (μL)	1400	1100	900	700	600
10% SDS (μL)	2	2	2	2	2

* NaCl: sodium chloride, Tris-HCl: 2-Amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride, SDS: sodium dodecyl sulfate

- Application of the gene probe: 90 μL hybridization buffer are added to 10 μL of the gene probe. After mixing this solution properly, 10 μL are applied to each slide well. Any possible source of light should be avoided. All the following steps were the gene probe is present were carried out away from light.
- Hybridization step: The slides with the gene probe are inserted in the 50 mL tube which contains the hybridization buffer solution. Then they are placed in a preheated water bath to 46°C. The duration of hybridization is from at least 1.5 h up to 72 h.
- Washing procedure: A washing solution is prepared. The selected formamide concentration also sets its composition. The detailed composition for each formamide concentration used is given in Table 8. The hybridized slides are transferred into a tube containing this washing buffer. Then, inside a water bath, incubation for 15 min at 48°C takes place. Afterwards, slides are dipped into deionized water for 5 min and finally dried at 46°C.

Table 8: Composition of the washing buffer corresponding to the formamide concentrations applied during the hybridization.

Formamide concentration	20%	25%	35%	45%	50%
5 M NaCl (μL)	2150	1490	700	300	180
1 M Tris-HCl (μL)	1000	1000	1000	1000	1000
0.5 M EDTA	500	500	500	500	500
10% SDS (μL)	50	500	50	50	50

* EDTA: Ethylenediaminetetraacetic acid

- Counterstaining: After all the above mentioned steps, the slides must be stained. The DNA stain 40, 60 – diamidino – 2 phenylindol (DAPI) is used. The reason is to determine the positive fraction of the slide out of the total DAPI count. Hence, 10 μL of DAPI are added to each well followed by staining for 10 min at 4°C in the dark. Then the slides are dipped in pure water and dried at 46°C. Clean cover slides are finally placed and carefully glued on the slides. The slides are ready for microscopy.

After the aforementioned preparation steps, the slides were examined by laser scanning microscopy with a Zeiss AXIO Imager M2 microscope (Carl Zeiss Microscopy GmbH, Germany). AxioVision Rel. 4.8 microscope software was used.

4.4 Theoretical estimation of the methane potential

There are different ways to estimate theoretically the methane potential from a certain biogas feedstock. In the present work this estimation was carried out with two ways. Both ways are described below. The potential gas yield calculation is based on the volatile solid concentration of the used substrate.

4.4.1 Biomethane Potential Test (BMP)

Generally, the aim of a biomethane potential test (BMP) is the determination of the biochemical methane potential and the degradation profile of the used inoculum and substrate.

In the present work Bioprocess™ automatic methane potential test system (AMPTS II, Bioprocess Control AB, Sweden) was used. The BMP assays were carried out in triplicate. Cellulose was used as a standard. The system employed 15 bottles, each of 500 mL total volume. Working volume was 400 mL. In this batch digestion system (Figure 11) each bottle was individually mixed by mechanical agitation. Interval, speed and rotation directions were adjustable. Via a large heated water bath the temperature was held constant at 37° C. A calculated quantity of inoculum and substrate was added to each bottle. The bottles used as a standard contained cellulose and a specific amount of inoculum whereas the control bottles contained only the inoculum.

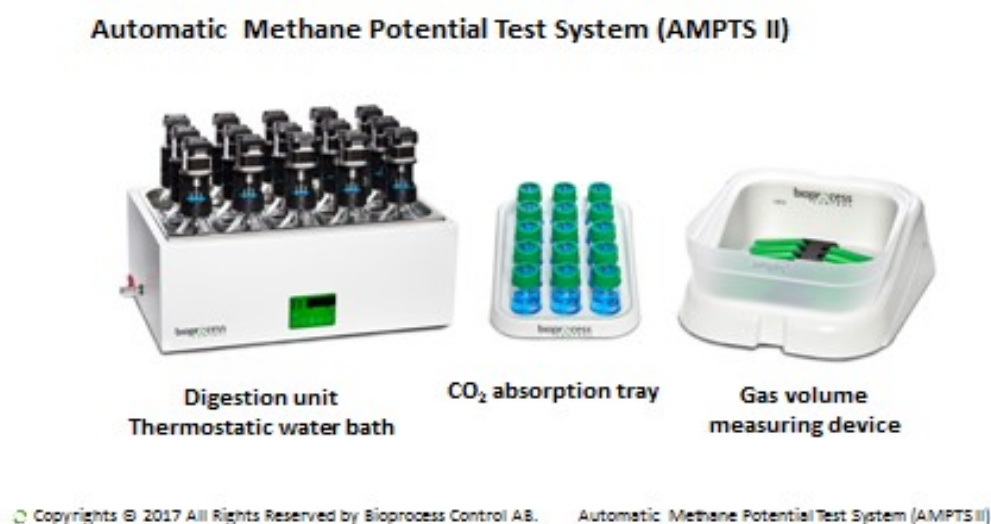
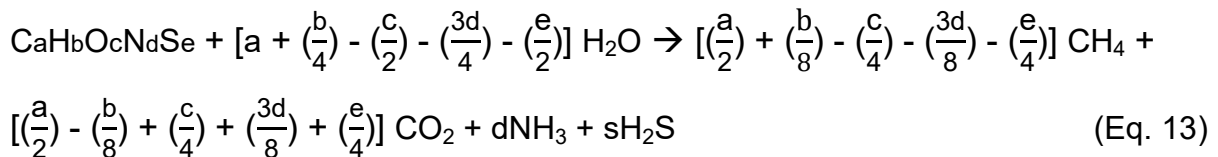


Figure 11: Main units of the system AMPTS II, Bioprocess Control AB, Sweden.

The inoculum to substrate ratio was set to 2:1. The final level in each bottle was 400 mL and de-ionized water was added when needed. A carbon dioxide absorption unit was following the main unit. Carbon dioxide and other trace gases were removed by passing through trap bottles filled with a 3 M sodium hydroxide solution (NaOH). The last unit of this BMP system was the flow cell array and data acquisition (DAQ) unit. It is a flow measurement device working under the principle of liquid displacement and buoyancy. When approximately 10 mL of upgraded gas were accumulated, the cell was opened. Gas was released. For every opening time, pressure and temperature were recorded on a bespoke software package. The BMP assays ran for 30 days. This system was described in detail by Wall et al., (2013).

4.4.2 Buswell's formula

Through ultimate analysis, the chemical composition of a waste can be known and the methane quantity can be predicted (Achinas & Euverink, 2016). Buswell & Hatfield (1936) proposed a formula which later along with Muller (Buswell & Muller, 1952) it was modified. This formula is outlined in Equation 13 below.



Once the element's coefficients are known, via this balanced reaction of water uptake, the production of methane can be calculated. However this formula assumes that the feedstock is consisted by only these elements. Moreover, since neither non-degradable material nor energy demand of the microbes are considered, the results indicate the maximal methane potential and they are often much too optimistic (Murphy et al., 2013).

4.5 Reactor start – up

In order to accomplish successfully the continuous operation of the experimental systems some steps were followed beforehand. All the required tubing was connected. Pumps were arranged and tests for their flow rate were carried out. The feeding tanks as well as the output tank were connected to system. All the measuring probes were calibrated and adjusted to the main reactor. All the equipment was aligned. Finally, several controls were made to ensure that the reactors and the overall experimental equipment are liquid and gas tight.

In order to start the continuous operation both reactors were inoculated with digested sewage sludge. Sparging with nitrogen (N₂) gas was used to establish anaerobic conditions to both feeding tank and the reactors. After a lag phase of 24 to 48 hours the feeding of the system started according to the set timers. Each reactor was fed 4 times per day. The experimental system was maintained in a daily basis and several

parameters were recorded manually. Input and output levels were noted along with the produced volume and composition of biogas. Samples were taken from the feeding tank (accounting for the input of the CSTR) and from the CSTR (accounting for the effluent of the CSTR). Several sampling ports were facilitating the control of the homogeneous content and the sufficient agitation of the reactors. The effluent itself was also collected for analysis. The samples were mostly analysed on the same day of sampling. Otherwise, samples were frozen up to the time of analysis so as to minimize microbiological decomposition of the organic content.

One reactor (CSTR₁) was used as control reactor operated throughout the experiment without hydrogen injection while the second one (CSTR₂) was used for upgrading biogas. The experimental configuration was operated under various operating conditions. The operation period was separated into phases to facilitate the description of the experiments. Each phase is characterized by different parameters. However, the main parameter examined was the biogas and methane volumetric yield expressed in L g⁻¹ VS fed.

5. Results and discussion

5.1 Introduction

The present work focuses on biological methanation with hydrogen utilization in the anaerobic digestion process. In order to evaluate this, three experimental systems were installed and operated in a continuous way. During these experiments the following steps took place:

- design of a stable process of treating sewage sludge for the production of biogas
- monitoring of the treatment system's performance
- supply of hydrogen to the anaerobic digester for *in – situ* biogas upgrading
- implementation of an *ex – situ* biogas upgrading process for hydrogen and carbon dioxide utilization, independent on biomass availability
- evaluation of the system's response in the application of different operational conditions.

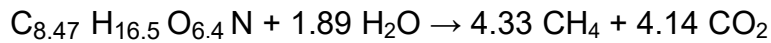
In the following chapter main results are presented and discussed. Initially, the theoretical calculations are presented followed by the results of continuous operation of the first experimental system. Then the performance of the second experimental system is presented. Afterwards, the configuration and the performance of the *ex-situ* methanation system is given. Moreover, a comparison between the different systems is presented. Finally, the overall performance is compared with similar studies and literature reports.

5.2 Theoretical maximum biomethane potential (BMP_{th}) and substrate chemical oxygen demand equivalent (COD_{th})

Elemental analysis was carried out for samples representing the substrate treated from the experimental anaerobic system. This analysis led to the stoichiometry of the sludge. Knowledge of the chemical composition of the sewage sludge led to the calculation of the theoretical maximum methane yield of the system (Table 9). Based on the stoichiometric equation the obtained yield was $413.2 \text{ L CH}_4 \text{ kg VS}^{-1}$ with a

methane concentration of 51.2%. It should be noted that for the calculation, sulphur (S) is considered to be negligible. Also it is assumed that volatile solids are consisted of C, O and H. The contribution of Nitrogen (N) is assumed to be negligible.

Table 9: Theoretical calculation of maximum methane potential and methane concentration using the Buswell Equation (based on Murphy et al., 2013).



$$234.38 \text{ g mol}^{-1} + 34.02 \text{ g mol}^{-1} \rightarrow 69.28 \text{ g mol}^{-1} + 182.16 \text{ g mol}^{-1}$$

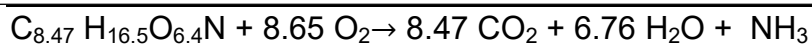
$$1 \text{ kg VS} + 0.14 \text{ kg } H_2O \rightarrow 0.295 \text{ kg } CH_4 + 0.77 \text{ kg } CO_2$$

$$\text{Density } CH_4: 0.714 \text{ kg m}^{-3} \quad \text{Density } CO_2: 1.96 \text{ kg m}^{-3}$$

Maximum theoretical biomethane potential equates to 413.2 L CH_4 kg VS^{-1} with a volumetric concentration of 51.2 Vol.-% CH_4 .

Since in theory the volatile solids of the used sewage sludge are expressed as $C_{8.47}H_{16.5}O_{6.4}N$, the theoretical COD equivalent could be also derived. In Table 10 the calculation is given.

Table 10: Theoretical calculation of COD equivalent expressed in g COD g VS^{-1} .



1 mol of sludge requires 8.65 moles of oxygen for total oxidation

$$234.38 \text{ g mol}^{-1} \text{ VS require } 276.8 \text{ g mol}^{-1} \text{ of oxygen}$$

$$1 \text{ g VS for total oxidation equates } 1.18 \text{ g of oxygen}$$

The theoretical COD equivalent results in a value of 1.18g COD g VS^{-1} .

5.3 Biomethane Potential Test Performance

A 30 day bio-methane potential (BMP) test was carried out in triplicate. A total methane yield of $294.2 \pm 7.7 \text{ L CH}_4 \text{ kg VS}^{-1}$ was obtained, which is 71.2% of the theoretical biomethane potential calculated in Table 10. Approximately 95% of the total methane production was obtained during the first 20 days of operation. This verified the findings about the optimum hydraulic retention time of the conducted continuous experiments.

5.4 Performance of the 1st experimental system (CSTR₁)

The first continuous experiment was an anaerobic continuous stirred tank reactor (CSTR) treating sewage sludge. No hydrogen was used in this system. Several operational conditions were applied in order to get a comprehensive idea about the waste's behaviour. Once the optimum conditions were determined the reactor was used as a control reactor for the second experimental system.

Throughout the continuous operation of the first treatment system (CSTR₁) three points were selected for representative sampling. The sampling points were the feeding tank (corresponding to the input of the CSTR), the contents of the CSTR (corresponding to the output of the CSTR) and the effluent. Their locations are shown in Figure 12.

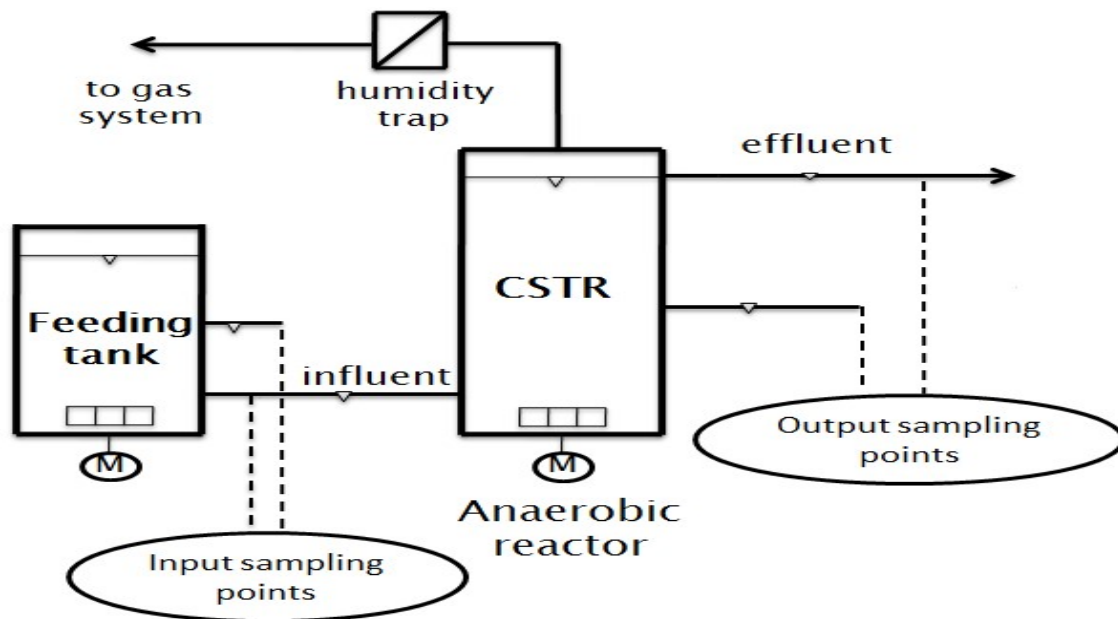


Figure 12: Schematic indication of the sampling points on the configuration.

5.4.1 Phase #1

In order to start the treatment system, the reactor was inoculated with digested sludge, which provided the methanogens required for biogas production. No pH control was required to allow the system to adapt to the introduction of sewage sludge. Start – up phase lasted for approximately 20 days. For the first three days of operation, the reactor was not fed with substrate, but a small amount of biogas was produced from residual substrate contained in the inoculum. Once this residual amount was almost consumed, continuous feeding of the system was started. Phase #1 lasted for 30 days. During this period the system adapted to the new operational conditions. Gas production was relatively stable and the average pH value was 7.27.

As shown in Figure 13, hydraulic retention time (HRT) was 28.8 days. Biogas production rate quickly stabilised and the organic loading rate (OLR) was $0.8 \text{ g VS L}^{-1} \text{ d}^{-1}$. The obtained methane yield was $0.178 \text{ expressed in } \text{L}_{\text{CH}_4} \text{ gVS}_{\text{fed}}^{-1}$. The solid concentration of the input was in average 34 g TS L^{-1} . The produced biogas had an average composition of 64% CH_4 .

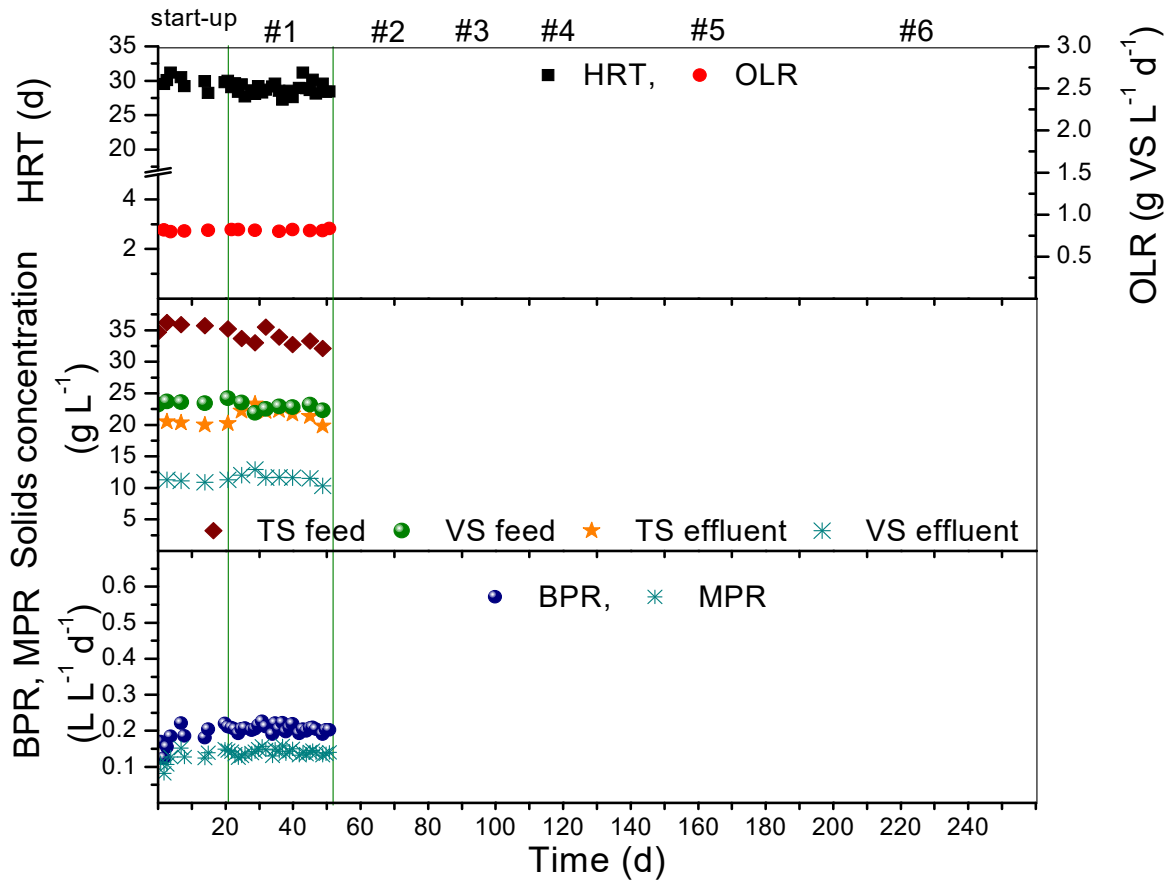


Figure 13: Performance of CSTR₁ system during start-up and phase #1. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

5.4.2 Phase #2

In Phase 2 (days 51 – 86), the reactor was operated with an OLR of $0.98 \text{ gVS L}^{-1} \text{ d}^{-1}$, and a HRT of 25.5 days. A fault to the stirring system meant that no feed was added to the reactor between days 56 and 60, which resulted in reduced biogas production, as seen in the shaded area in Figure 14. The mechanical stirring system was replaced with a magnetic system, and feeding was resumed and restored on day 61.

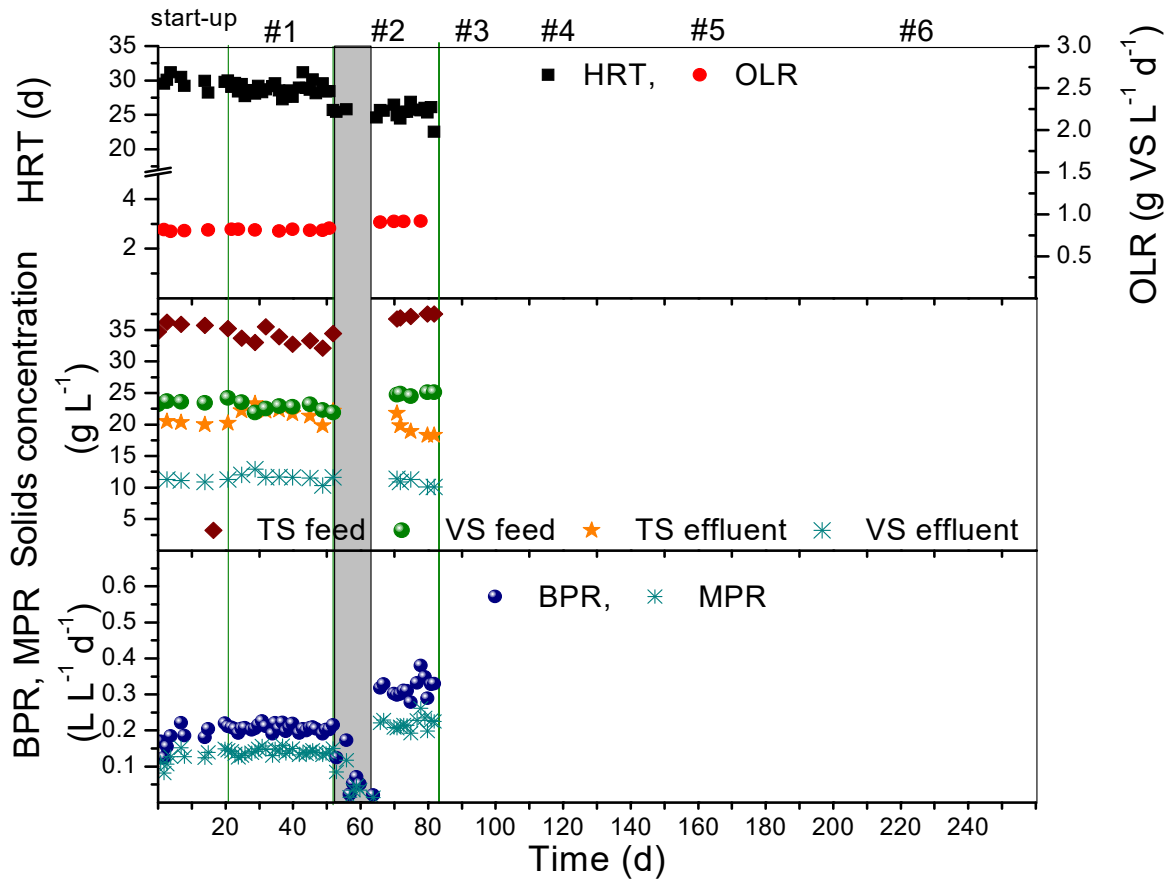


Figure 14: Performance of CSTR₁ system until the 2nd operational phase. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

As shown in Figure 14, the system managed to cope with this disorder when the organic loading rate was increased to the desired level. No pH adjustment was required. The solids concentration in the reactor was slightly higher, attributable to the increased OLR/decreased HRT. The COD removal efficiency in Phase 2 was 64%. The average biogas production rate was $0.309 \text{ L L}_{\text{reactor}}^{-1} \text{ d}^{-1}$. The methane content in the biogas remained in the range of 60 to 65%.

5.4.3 Phase #3

In the following figure (Figure 15) the performance of the system including phase 3 is presented. This phase lasted until the 108th day of system's operation.

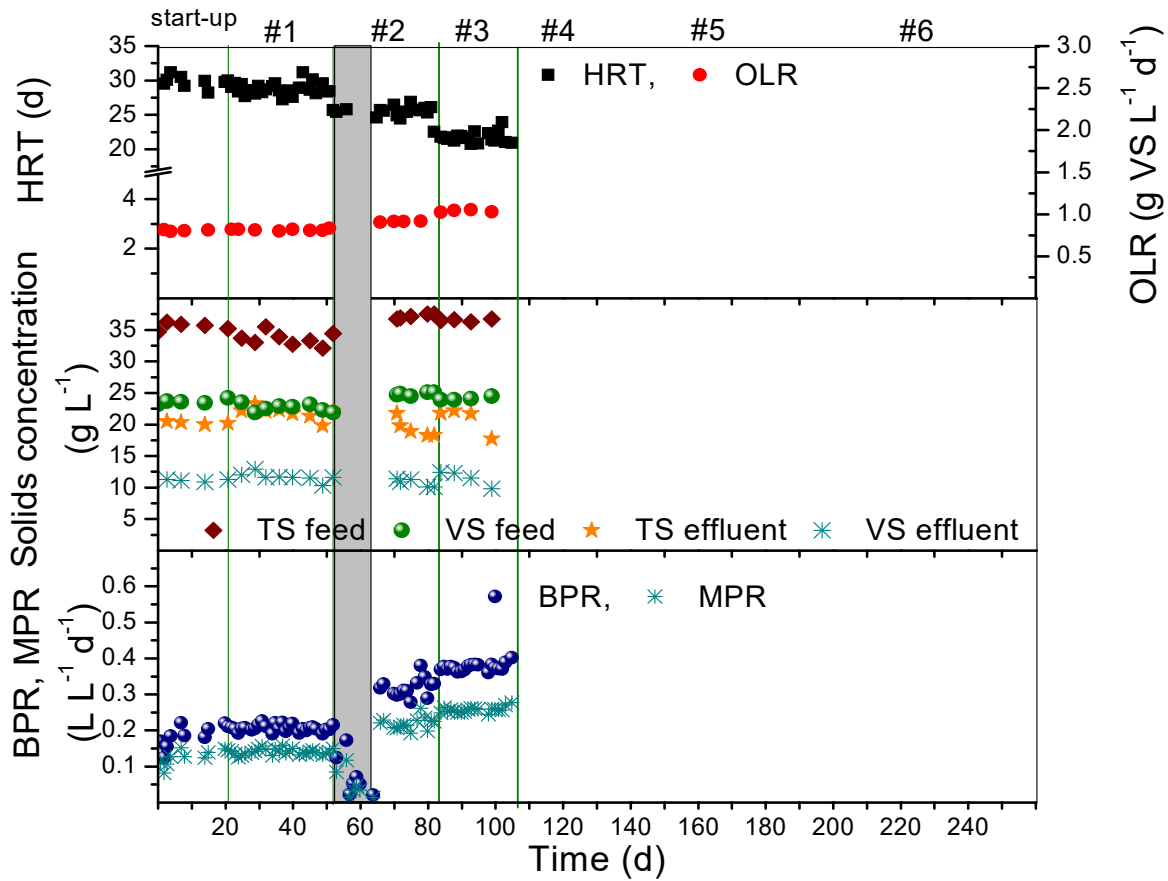


Figure 15: Performance of CSTR₁ system until the 3rd operational phase. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

During this phase, a further decrease of the hydraulic retention time was implemented. By increasing the amount of substrate fed into the system, HRT reached the value of 21.6 days. Organic loading rate was increased to 1.13 g VS L⁻¹ d⁻¹ and biogas production was increased as well. Higher biogas production rate was also observed to 0.334 L_{CH₄} g VS_{fed}⁻¹ and the percentage of methane was above 67%.

5.4.4 Phase #4

For the next 36 days of operation hydraulic retention time was further decreased (Figure 16). Having an HRT of 19.6 days and an organic loading rate of 1.27 g VS L⁻¹ d⁻¹ led to the highest biogas production until that point. Total solids concentration at the feeding stream was 37.5 g kg⁻¹ with 66.3% being volatile solids. Solids degradation reached a level constantly higher than 65%.

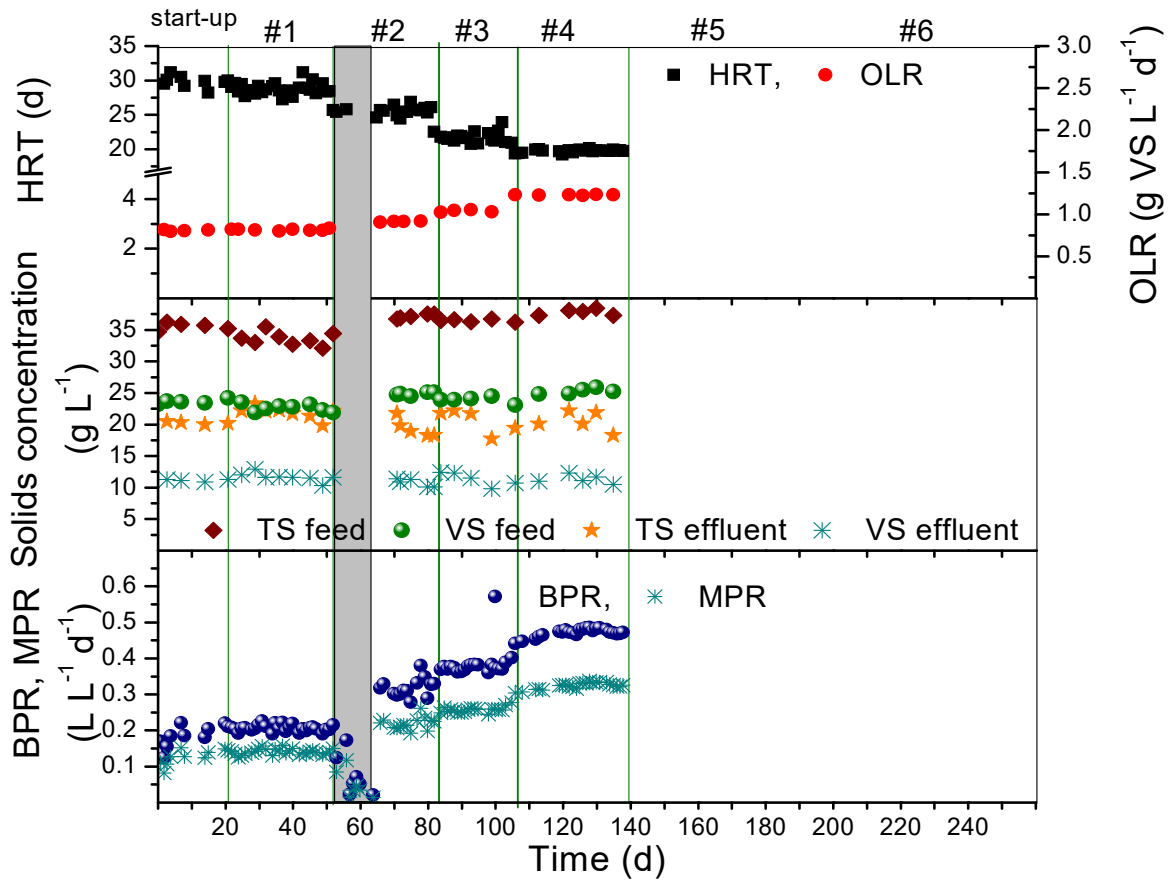


Figure 16: Performance of CSTR₁ system until the 4th operational phase. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

Furthermore, during this operational phase biogas and methane production rate were quite stable in comparison to the previous applied conditions. The pH was slightly increased to an average value of 7.35. Finally this phase presented the highest methane percentage with an average of 68.8%.

5.4.5 Phase #5

The following phase lasted for 53 days. During the fifth operational phase the flow rate of the substrate fed into the system was adjusted once more. The organic loading rate was increased to $1.34 \text{ g VS L}^{-1} \text{ d}^{-1}$ and consequently the hydraulic retention time also reached the level of 18.1 days.

As it can be seen in Figure 17, the system did not correspond as well as previously to these new conditions. A thick layer, similar to foam, was observed on the top of the reactor.

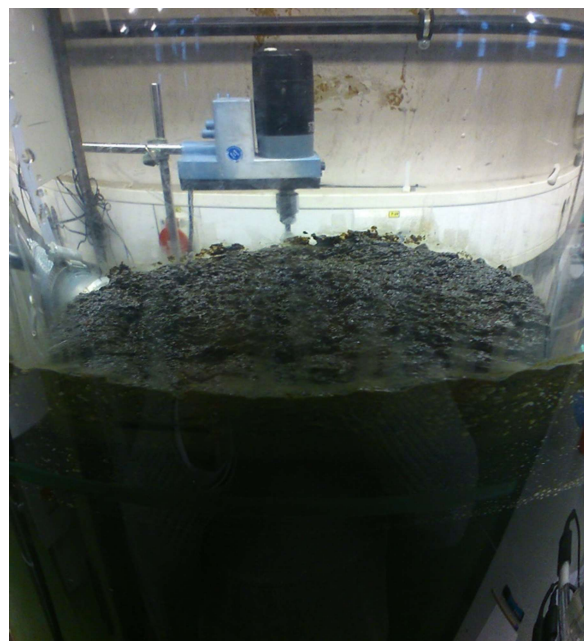


Figure 17: Thick layer created during operational phase 5 at CSTR₁ system.

The output of the system was in some extent obstructed. As Figure 18 illustrates, the gas production rate initially presented a sudden drop. The overall picture of the system was not the same. Furthermore, the pH values as well as the produced gas volume presented many fluctuations.

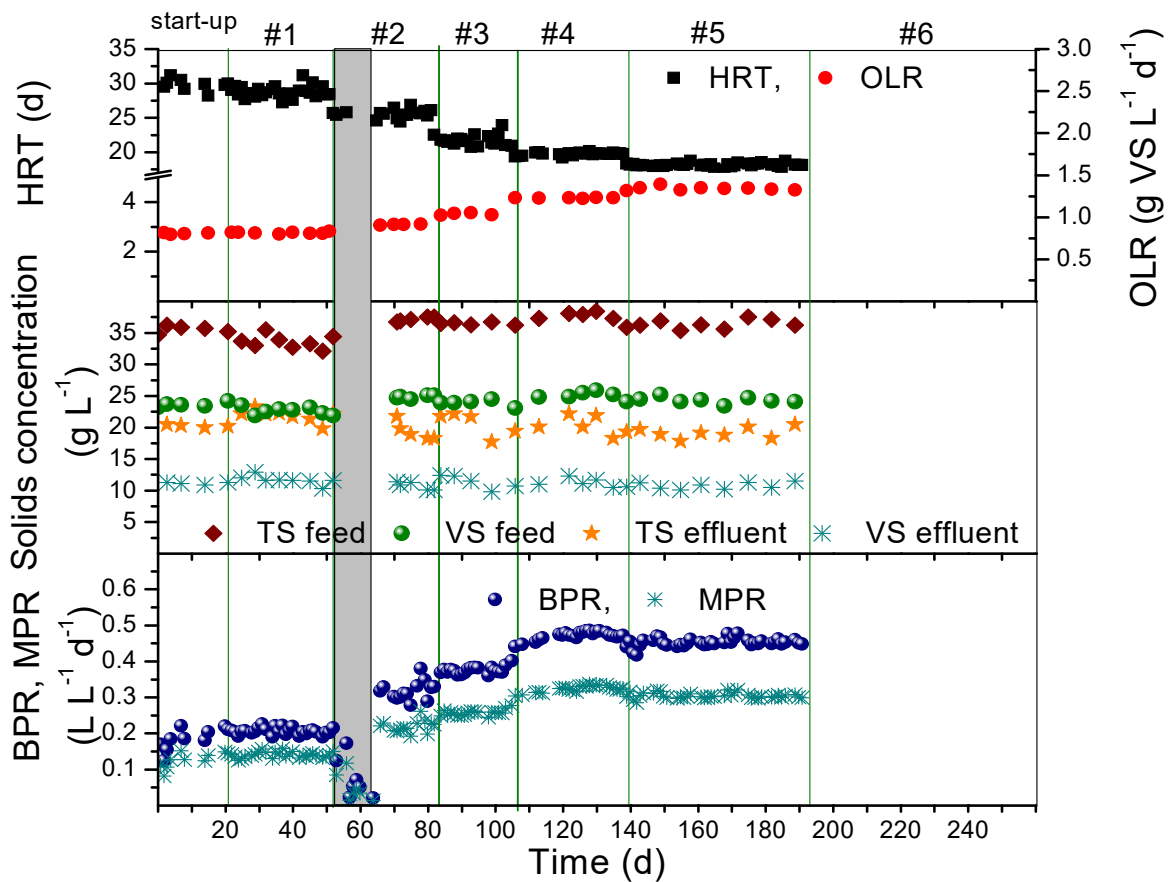


Figure 18: Overall performance of CSTR₁ system until the 5th operational phase. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

After about 10 days the system gained a much more improved picture. The surface of the reactors' content had no thick layer and the fluctuations of the values of the main parameters measured were back to normal. However, as regards the overall performance of this operational phase the obtained yields of biogas and methane were lower than the previous phase. The achieved values were $0.337 \text{ L}_{\text{biogas}} \text{ g VS}_{\text{fed}}^{-1}$ and $0.229 \text{ L}_{\text{CH}_4} \text{ g VS}_{\text{fed}}^{-1}$, accordingly.

5.4.6 Phase #6

During the last operating days of this continuous system biogas recirculation was evaluated. The duration of this phase was about 60 days.

Initially, the main parameters were restored to the same values as in the fourth phase. Main performance results are shown in Figure 19.

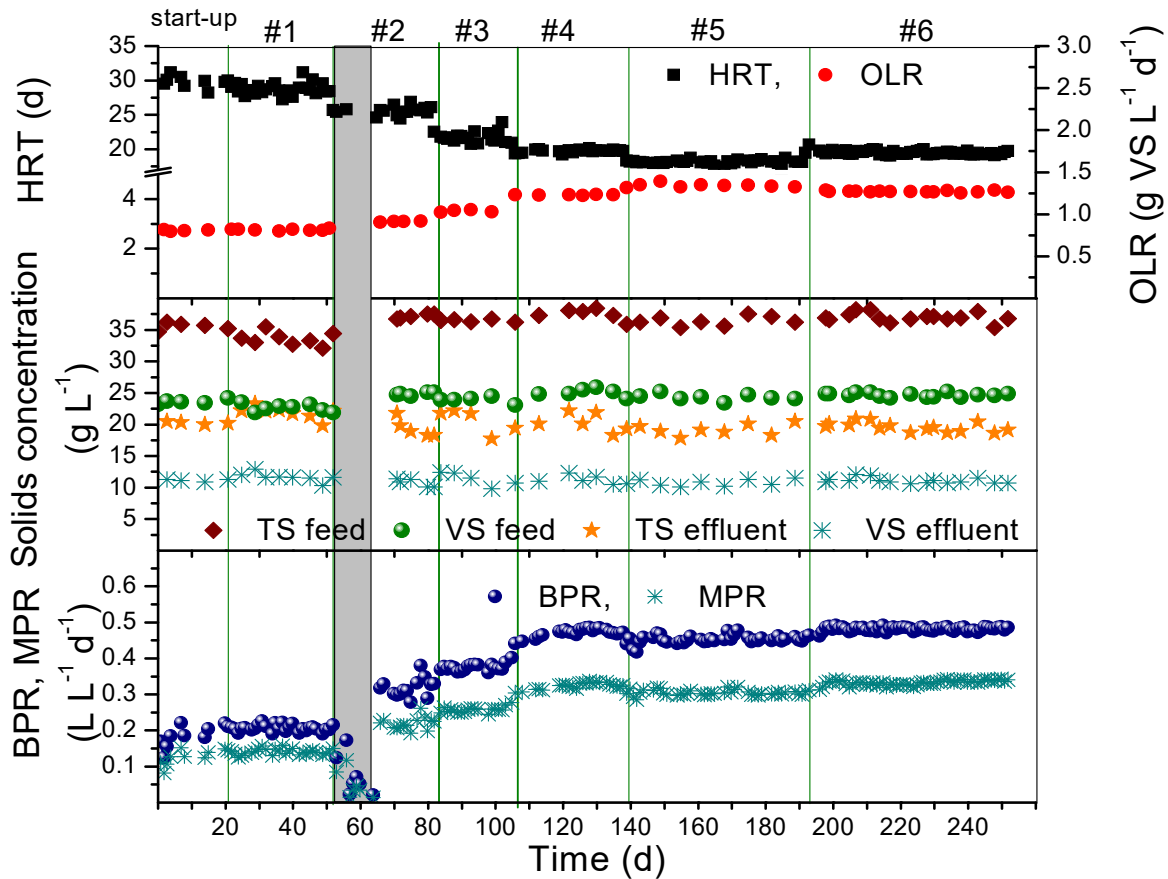


Figure 19: Performance of the CSTR₁ system. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

The produced biogas was collected in gas sampling bags (Restek GmbH). After the measurement of its composition, the collected gas was recirculated back into the system. The rate of the recirculation was around $0.45 \text{ L L}_{\text{reactor}}^{-1}$. After this step the total biogas production was calculated.

The hydraulic retention time was 19.6 days. The organic loading rate was $1.27 \text{ g VS L}^{-1} \text{ d}^{-1}$. During this phase it was observed that biogas recirculation slightly increased biogas and methane production rate. However the increase was not higher than 5%. The measured values were $0.379 \text{ L}_{\text{biogas}} \text{ g VS}_{\text{fed}}^{-1}$ and $0.262 \text{ L}_{\text{CH}_4} \text{ g VS}_{\text{fed}}^{-1}$, accordingly.

This phase was implemented as biogas recirculation could play a significant role during the second continuous experimental period. Therefore, in order to have a comparison, recirculation was evaluated.

5.4.7 Overall performance of experimental system CSTR₁

The different applied operational conditions along with the average values of the main parameters are summarized in Table 11. By adjusting the flow rate of the substrate, gradually decreasing HRT and consequently increasing OLR were implemented. In the last phase biogas recirculation was applied.

Table 11: Average values of the main parameters during each operational phase of system CSTR₁.

Parameter	Phase #1 (21–51d)	Phase #2 (52–86d)	Phase #3 (87–108d)	Phase #4 (109–144d)	Phase #5 (145–197d)	Phase #6 (198–257d)
						biogas recirculation
HRT (d)	28.8	25.2	21.6	19.6	18.1	19.6
Flow rate (L d⁻¹)	0.33	0.38	0.44	0.48	0.53	0.48
OLR (g VS L⁻¹ d⁻¹)	0.79	0.98	1.13	1.27	1.34	1.27
Biogas yield (L_{biogas} gVS_{fed}⁻¹)	0.259	0.299	0.334	0.372	0.337	0.379
CH₄ yield (L_{CH₄} gVS_{fed}⁻¹)	0.178	0.207	0.227	0.256	0.229	0.262

The solids concentration had a constant average giving the expected fluctuations. The VS percentage of the influent was about 67 - 68% of the total solids concentration and more than half of it was converted to biogas. That is the reason that biogas and methane yield both given in terms of L L⁻¹ d⁻¹ followed the increasing organic loading rate.

As presented in Figure 20, the performance of the system was mainly determined by biogas and methane production rate expressed in L g VS_{fed}⁻¹.

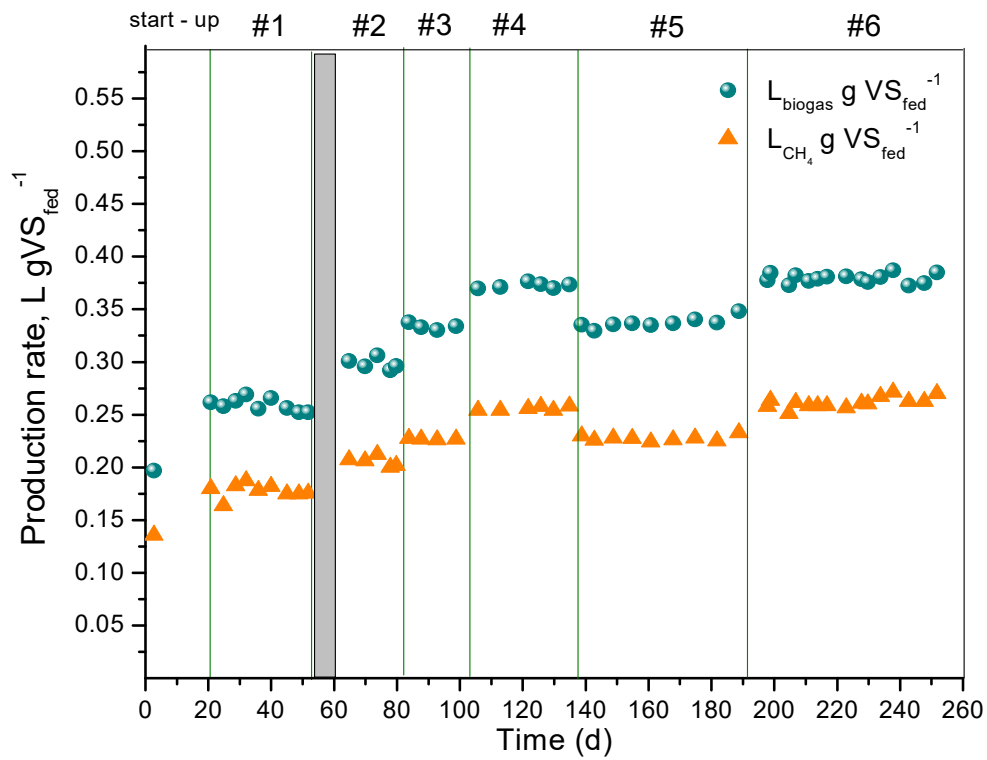


Figure 20: Time evolution of biogas and CH_4 production rate per g VS fed during each operational phase of system CSTR_1 .

Through this operation, a comprehensive idea about the waste's behavior and the process stability was obtained. After these modifications the optimum operational conditions were determined and applied to the next experimental system where hydrogen was introduced into the reactor.

5.5 Performance of the 2nd experimental system (CSTR₂)

In order to examine if biogas can be *in – situ* upgraded as well as how much can methane be enhanced a second system (CSTR₂) was set up. The system consisted of the feeding tank and a continuous stirred tank reactor similar to the first system, but with the option to inject hydrogen.

Hydrogen entered the liquid phase through ceramic gas diffusers (Figure 21) which produced very fine bubbles which give a very high surface area. Additionally, the diffusers were located as close to the base of the tank as practicable to ensure as long a contact time as possible between. Gas - liquid mass transfer is crucial and determines the available substrate for methanogens (Pauss et al., 1990).

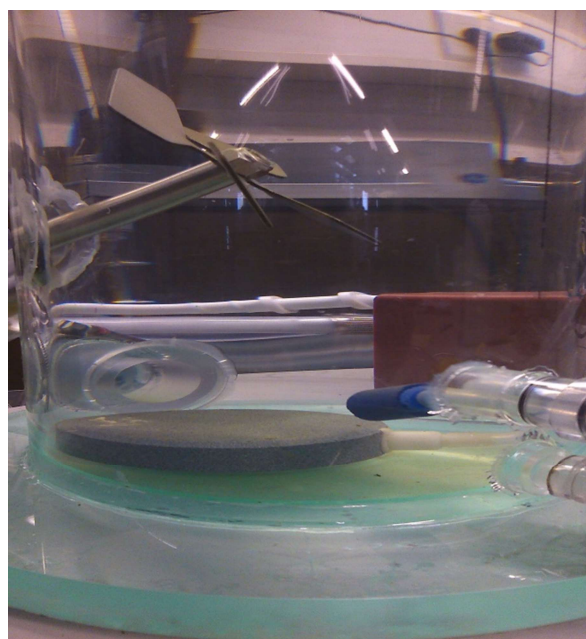


Figure 21: Ceramic diffusers for hydrogen addition.

The experiments were conducted at mesophilic temperature (37° C). The sampling points were the same as the first system (shown in Figure 12) and corresponded to the same streams of the process.

During this operation different conditions were applied such as different H_2 flow rates as well as biogas recirculation. The system was installed and operated for a period of about 7 months. According to the different conditions implemented to the system its performance is presented per operational phase.

5.5.1 Phase #1

In order to start the treatment system, the reactor was inoculated with digested sludge, using effluent of the first system (CSTR₁) as the inoculum. No pH control was required since the inoculum was already adapted to the mesophilic anaerobic digestion process.

Additionally, no start – up phase was required and feeding of the reactor started on the second day of operation. Phase #1 lasted for 46 days. During this period the system adapted to the new operational conditions. The conditions implemented corresponded to the operational phase #4 of system CSTR₁. Gas production was relatively stable and the average pH value was 7.27.

As shown in Figure 22, HRT was 19.6 days and the OLR was $1.27 \text{ g VS L}^{-1} \text{ d}^{-1}$. Biogas production rate quickly stabilised and the obtained methane yield was $0.259 \text{ L}_{CH_4} \text{ gVS}_{fed}^{-1}$. This value was similar to the methane yield in the phase #4 of the control reactor where the conditions were the same. The average concentration of the input was 34 g TSL^{-1} , and produced biogas had an average methane content of 68.7%.

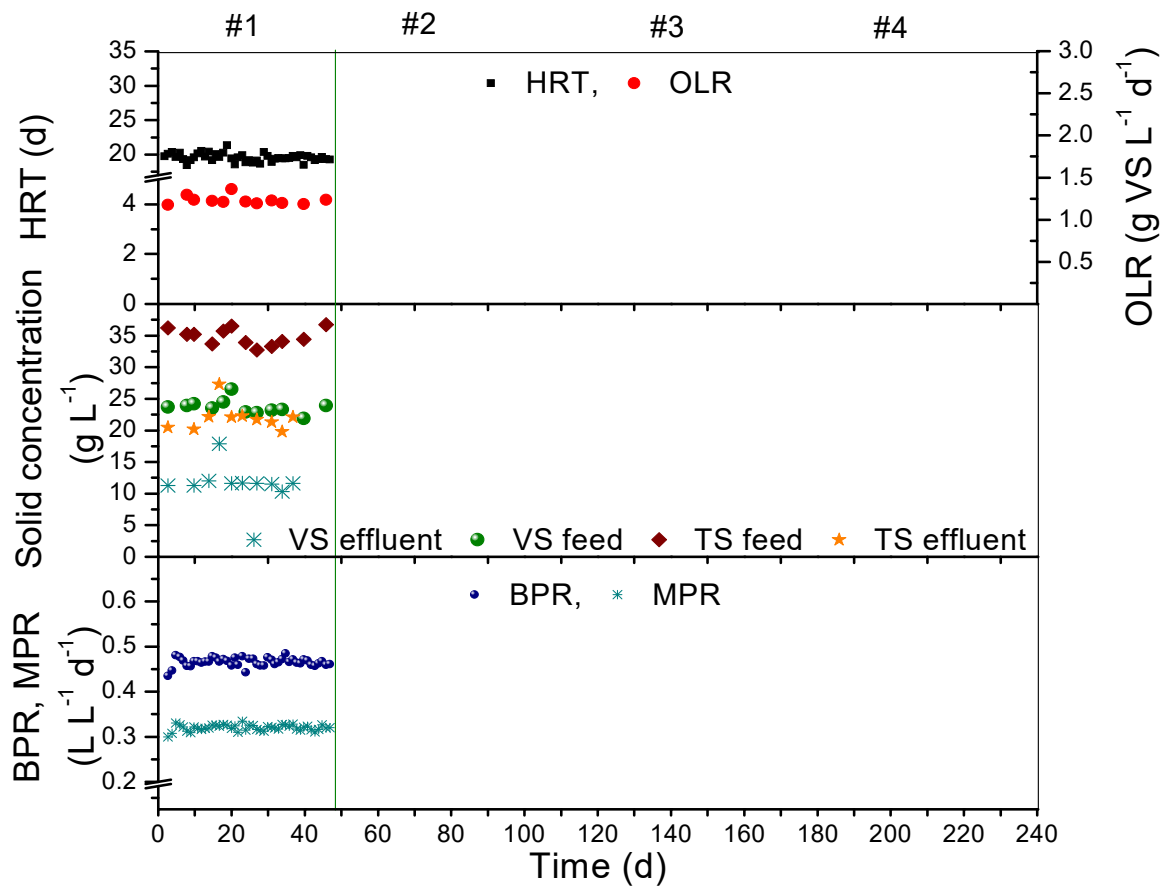


Figure 22: Performance of system CSTR₂ during start-up and phase #1. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

The purpose of this phase was to verify that the sewage sludge treatment performance of CSTR₂ is similar to the control reactor (CSTR₁), and that therefore comparable results will be obtained.

5.5.2 Phase #2

During the second operational phase, which lasted 56 days, hydrogen was injected into the anaerobic reactor. The implemented hydraulic retention time was kept the same since the influence of the increasing flow rate of hydrogen was evaluated. The organic loading rate of the system was $1.27 \text{ g VS L}^{-1} \text{ d}^{-1}$. As shown in Figure 23, biogas production rate remained almost unaffected by the continuous addition of hydrogen. However, methane production rate was $0.347 \text{ L L}^{-1} \text{ d}^{-1}$, approximately 9% higher compared to the control reactor fed only with sewage sludge.

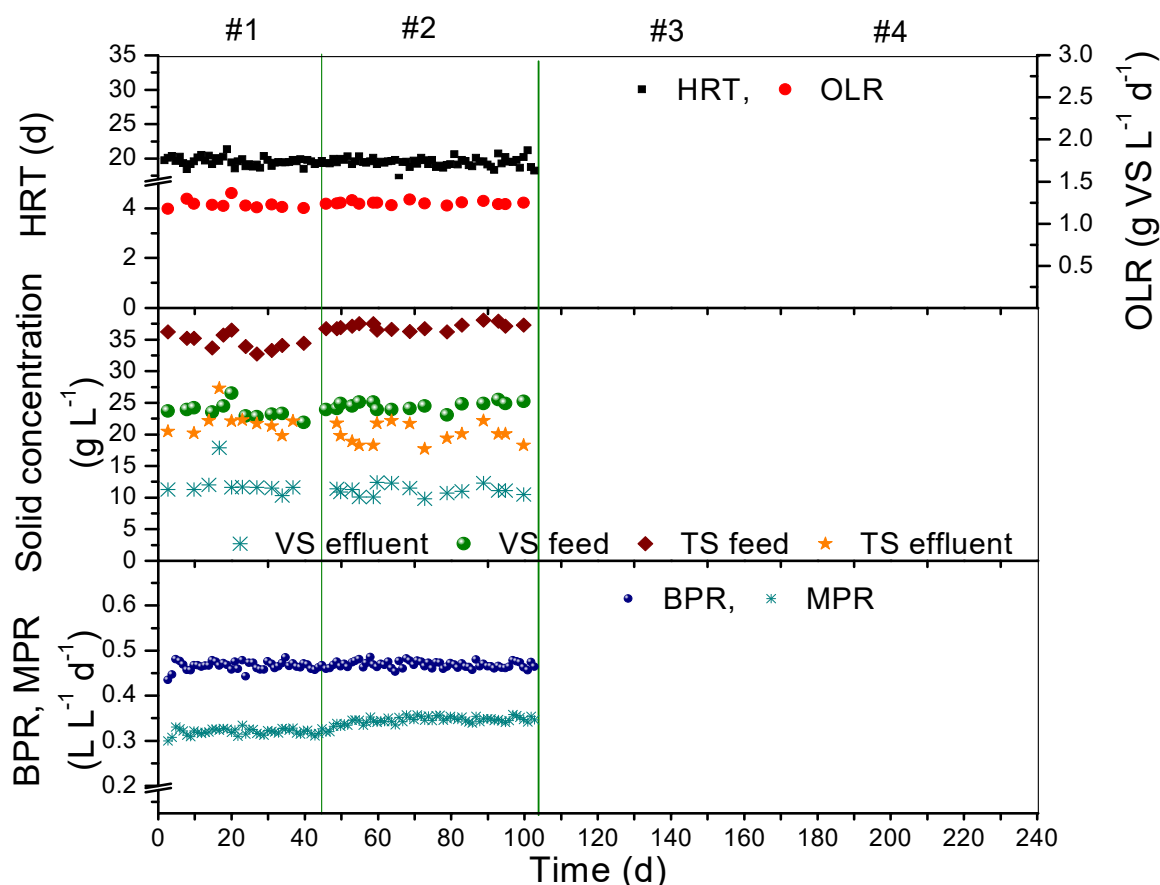


Figure 23: Performance of the system CSTR₂ during 2 operational phases. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

Furthermore, CO₂ content in the produced biogas decreased to a value of 18.5% whilst in the control reactor and during the first operational phase of this system the CO₂ content was 31.3%. Hydrogen was added to the system at a flow rate of 0.437 L L⁻¹ d⁻¹. The maximum tested hydrogen rate was meant to be tested during the next phase (#3). Hence a lower flow rate was chosen in order to evaluate the system's correspondence to hydrogen addition. The conversion of hydrogen was not complete, with hydrogen making up 7.6% of the produced biogas.

5.5.3 Phase #3

For the next 61 days of operation hydrogen flow rate inserted to the system was increased to 0.582 L L⁻¹ d⁻¹, a value corresponding to 4 times the volume of CO₂ production rate in the control reactor reflecting the stoichiometric ratio of H₂:CO₂ for CH₄ production given by the Sabatier's reaction (see Reaction 6). Main performance parameters are shown in Figure 24.

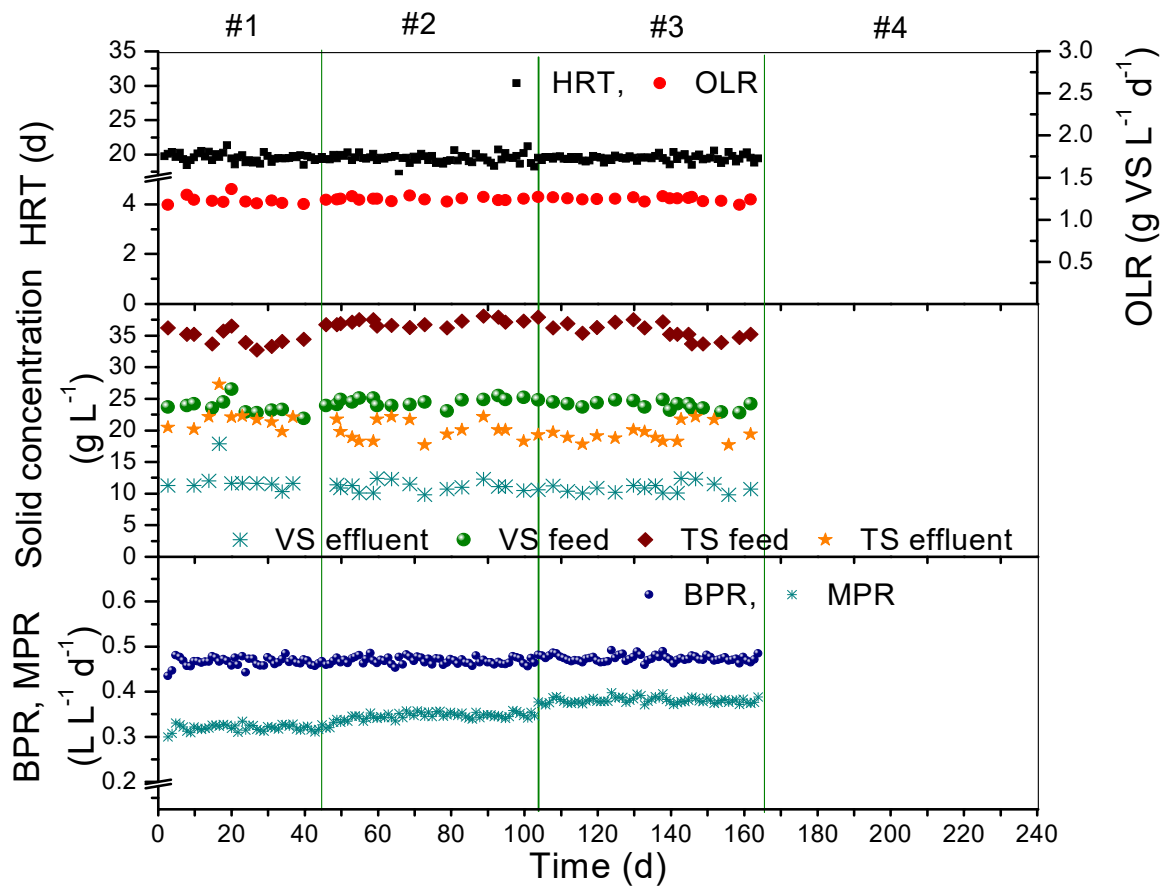


Figure 24: Performance of the system CSTR₂ during 3 operational phases. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

While hydraulic retention time was kept constant and solids concentration was similar to the previous phase, giving the expected fluctuations, the increased addition of hydrogen did not appear to influence biogas production rate. It was slightly increased to $0.474 \text{ L L}^{-1} \text{ d}^{-1}$. Methane production rate on the other hand was 9% higher than the previous applied hydrogen flow rate and around 16.3% higher compared to the control reactor. Produced biogas had an average composition of 80.3% CH₄ with CO₂ being further reduced to 15.5%. Hydrogen was not fully consumed. Still the unutilized percentage was decreased.

5.5.4 Phase #4

For the next 60 days of operation biogas recirculation was implemented. In the sense that biogas as well as the remaining hydrogen were in mixture collected in a gas bag and recirculated again into the reactor's liquid phase. Hydraulic retention time remained 19.6 days. Hydrogen flow rate corresponded to H₂:CO₂ volumetric

ratio of 4:1 as previously. In the following Figure 25 the performance of the system including overall operation is presented.

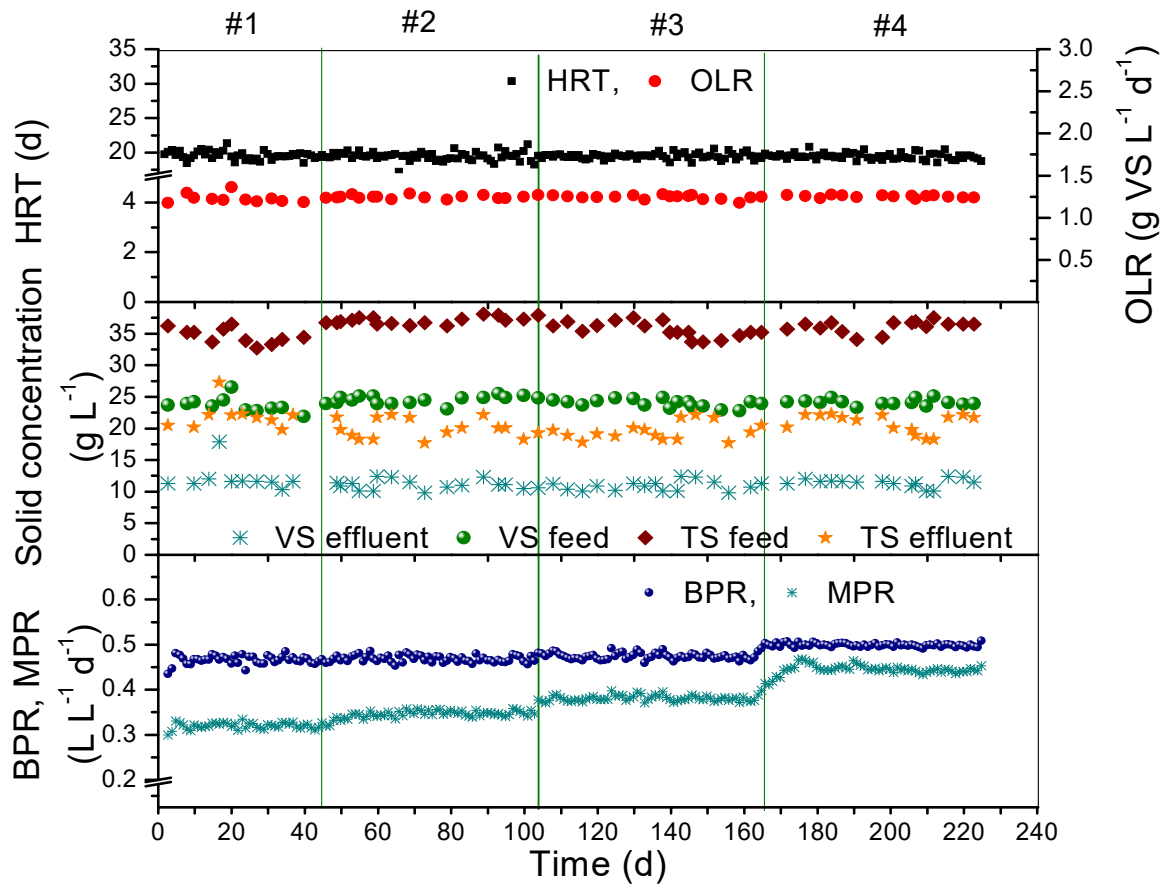


Figure 25: Overall performance of system CSTR₂. Time evolution of biogas and CH₄ production rate (BPR, MPR), solids concentration (TS, VS), organic loading rate (OLR), hydraulic retention time (HRT).

During this phase of operation the system gave the highest performance. Hydraulic retention time was stable throughout the whole operation. The solids concentration was no different than the control reactor. Throughout the whole operation the volatile solid (VS) concentration was not influenced by the addition of hydrogen. Biogas production rate was about 7% higher and CH₄ production rate more than 30% compared to the control reactor and the pre – H₂ phase (#1). Also, compared to phase #3, there was also a significant increase of 5% and 17% to the biogas and methane production rate, respectively. These results indicate the positive effect of biogas recirculation on the biological methanation process. Finally, biogas composition was affected. CH₄ resulted in an average of 89% with a maximum of 95.2%.

5.5.5 Change of metabolic products and pH value

During the operation of CSTR₂ the effect of hydrogen on volatile fatty acids degradation was evaluated. Samples were collected during each phase of operation and analysed for VFA concentration using a GC. The results of these analysis, expressed in g L⁻¹, are given in Figure 26 below.

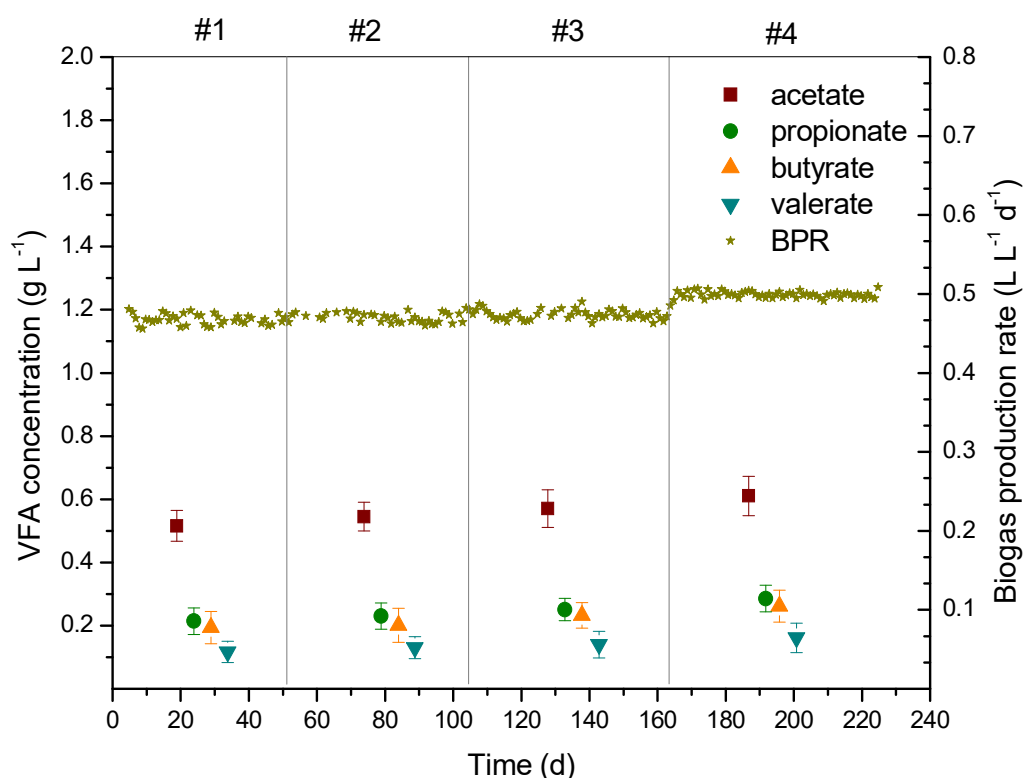


Figure 26: Time evolution of average VFA concentration and biogas production rate of system CSTR₂.

Figure 26 illustrates the average value of the main metabolites along with the standard deviation. Several samples were tested for VFA concentration. However there were no significantly different values for each operational phase. For that reason an average value for each phase is shown.

Acetate was the main metabolite detected in the liquid phase with its concentration ranging between 0.5 and 0.62 g L⁻¹, while the rest of volatile fatty acids had a concentration < 0.3 g L⁻¹. The total volatile fatty acids concentration (tVFA) had an average of 1.128 g L⁻¹.

Propionate, butyrate and valerate, during the pre – H₂ phase (phase #1) as well as the next two ones (phase #2 and #3), they had similar degradation trends. They showed a minor upward trend of about 4 – 7%. While during phase #4 their concentration was further increased at a value of 13 – 14% higher. The values were 0.285 g L⁻¹, 0.261 g L⁻¹ and 0.16 g L⁻¹, for propionate, butyrate and valerate, respectively. Hence, the increased hydrogen flow rate as well as biogas recirculation where implemented without any obvious inhibition to be observed.

Regarding acetate, only in the last operational phase was any effect of increased hydrogen flow rate observed. This could be attributed to pH level which remained below 7.6 – 7.7. Once pH value was further increased acetate concentration followed to higher values. According to O' Flaherty et al., 1998, the optimal pH range for the growth of acetoclastic methanogens is between 7.0 – 7.5. For this reason, a slight inhibition of acetoclastic methanogenesis resulted in the increased levels of acetate during the last 60 days of operation.

The value of pH was closely observed in order to prevent any system instability. Methane formation takes place within a relatively narrow pH interval, from about 6.5 to 8.5 with an optimum interval between 7.0 and 8.0. The process is severely inhibited if the pH decreases below 6.0 or rises above 8.5 (Weiland P. 2010). Figure 27 highlights the effect of pH on biogas composition.

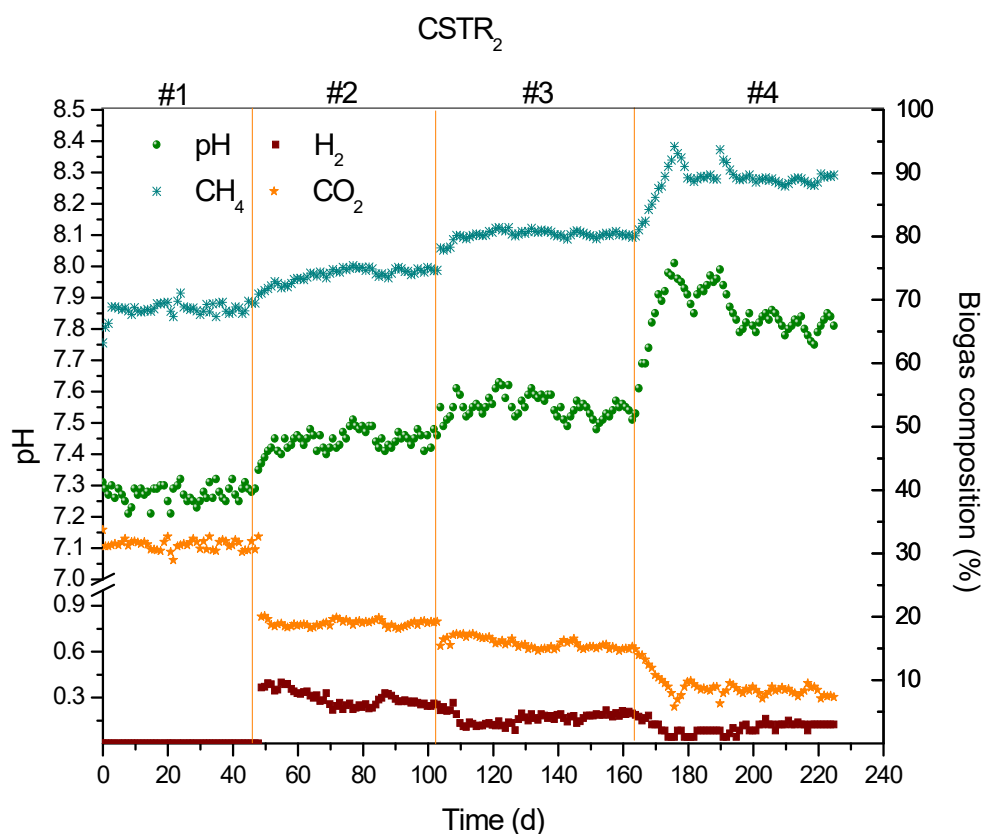


Figure 27: Composition of biogas and pH values during each operational phase of system CSTR₂.

The addition of hydrogen into the system resulted in an increase of pH. During the pre – H₂ phase (phase #1 of CSTR₂) the average pH value was 7.27. Once H₂ was injected pH start rising due to the consumption of bicarbonate. During phase #2 and #3, pH increased to averages of 7.44 and 7.65 respectively, which was in accordance with the increased conversion efficiency of H₂ to CH₄. During the last operational phase, CH₄ content in the produced biogas resulted in an average of approximately 89%, with a maximum of 95.2%, with pH increasing to an average of 7.85. The increase in pH did not result in any inhibition nor reduction of H₂ and CO₂ conversion to methane. Overall adaption of microorganisms to elevated pH values was indicated.

5.5.6 Overall performance of experimental system CSTR₂

During the continuous operation of CSTR₂ system four different operational conditions were implemented. *In – situ* biogas upgrading with the use of hydrogen was evaluated. The different applied operational conditions along with the average values and standard deviation of the main parameters are summarized in Table 12.

Table 12: Summary table of the average values of the main parameters during each operational phase of system CSTR₂.

Parameter	Phase #1 (0 – 46d)	Phase #2 (47-102d)	Phase #3 (103-164d)	Phase #4 (165-224d)
	(pre – H ₂)			Biogas recirculation
Biogas Production rate (L L⁻¹ d⁻¹)	0.465	0.469	0.474	0.499
Biogas Composition				
CH ₄ (%)	68.7 ± 0.8	73.9 ± 1.2	80.3 ± 0.7	89.1 ± 2.3
CO ₂ (%)	31.3 ± 0.8	18.5 ± 1.2	15.5 ± 0.8	8.8 ± 1.6
H ₂ (%)	–	7.6 ± 0.8	4.2 ± 0.7	2.1 ± 1.1
CH₄ production rate (L L⁻¹ d⁻¹)	0.319	0.347	0.381	0.445
Biogas yield (L_{biogas} gVS_{fed}⁻¹)	0.375	0.377	0.379	0.398
CH₄ yield (L_{CH₄} gVS_{fed}⁻¹)	0.259	0.277	0.304	0.356
pH	~ 7.27	~ 7.44	~ 7.55	~ 7.85

The system exhibited an increase of methane content without any significant inhibition. Gradually increased flow rate of hydrogen into the system result in enhanced percentage of methane.

As presented in Figure 28 the performance of the system was mainly determined by the biogas and methane production rate expressed in L g VS_{fed}⁻¹.

The use of the volumetric ratio of H₂:CO₂ in respect with the stoichiometric ratio of Sabatier reaction (see Reaction 6) gave an average of 0.304 L_{CH₄} g VS_{fed}⁻¹. Biogas production rate was slightly increased. However fluctuations of solids concentration can be responsible for that. During the last operational phase (phase #4), biogas recirculation was tested. The highest biogas yield was observed with an average value of 0.356 L_{biogas} g VS_{fed}⁻¹.

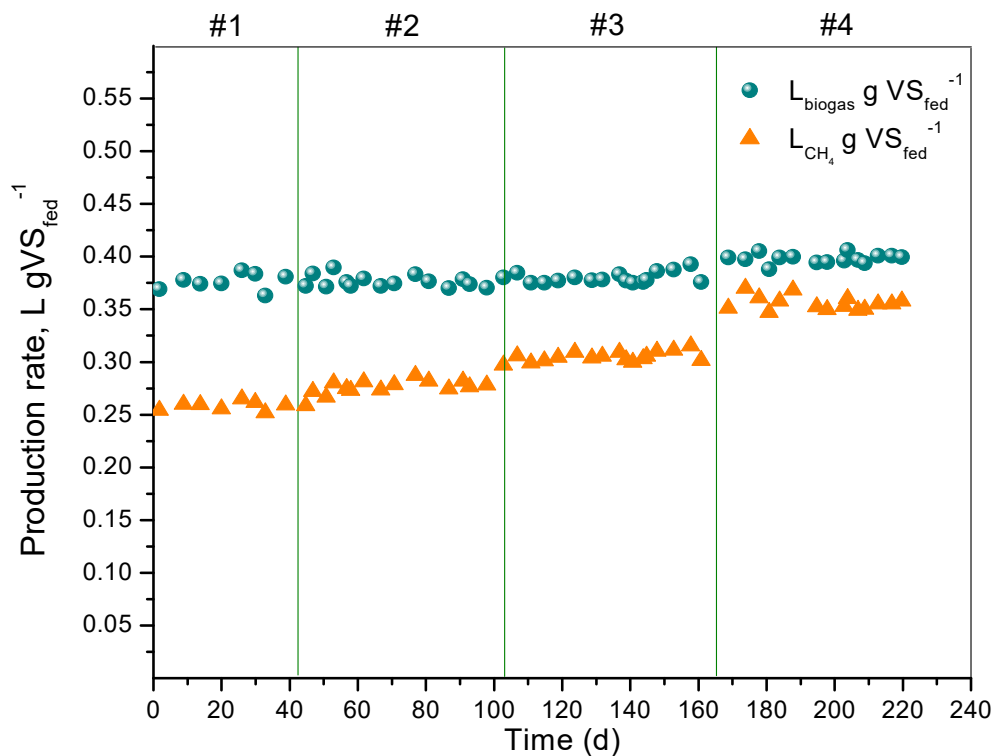


Figure 28: Time evolution of biogas and CH_4 production rate per g VS fed during each operational phase of system $CSTR_2$.

Biogas composition had 89.1% CH_4 with carbon dioxide concentration almost half as from the previous phase (phase #3). The pH values were increased. Acetate concentration followed. Nevertheless, microorganisms seemed able act to this higher values. The reported inhibition threshold of pH = 8 was not reached (O' Flaherty et al., 1998, Weiland P. 2010).

5.6 *Ex – situ* upgrading experimental system $CSTR_3$

After the *in – situ* biogas upgrading experimental period a different approach to biological methanation was tested. The previous system ($CSTR_2$) was able to treat organic waste and consume the added hydrogen. Microorganisms were adapted to these conditions. Hence, while keeping the same reactor under the same mesophilic temperature conditions (37° C), feeding of organic waste to the system was stopped.

A gas mixture of hydrogen and carbon dioxide as a sole substrate (gaseous) was added to the system. The volumetric ratio of the two components was 4:1 according to Sabatier's reaction (see Reaction 6). A simplified configuration of the system, from now on referred as CSTR₃, is given in Figure 29.

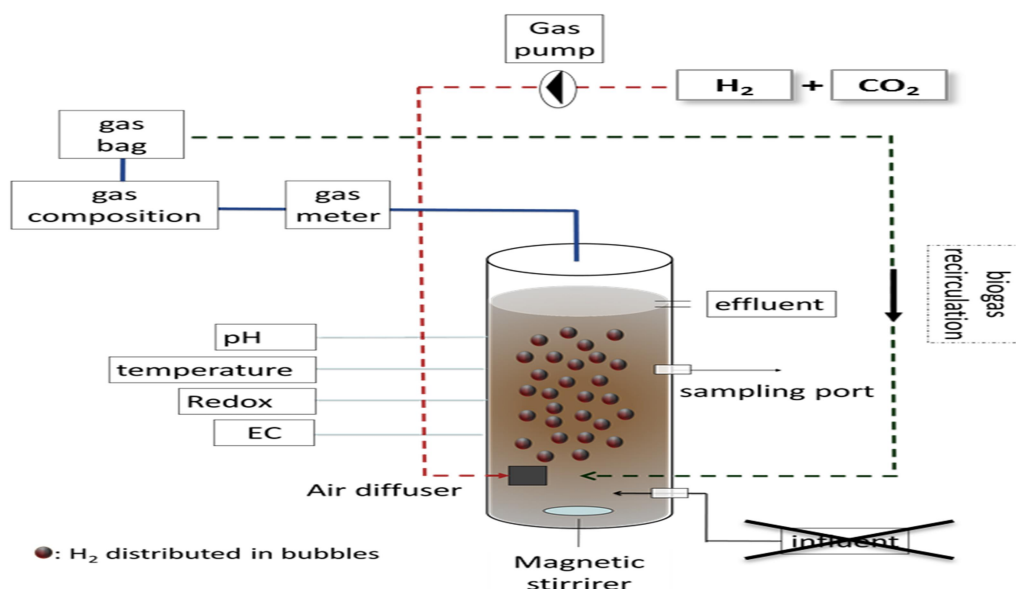


Figure 29: Schematic configuration of the 3rd experimental system (CSTR₃).

The examination of whether H₂ and CO₂ utilization can take place, independent on biomass availability along with the evaluation of process stability was the objective.

During this operation the feeding gas mixture consisted of 80%:20% hydrogen and carbon dioxide, respectively. The gas injection rate was set at 6.9 L d⁻¹ based on the control reactor's biogas production and composition. Hydrogenotrophic methanogenic activity in the inoculum was present, and consumption of the feed gas began immediately on start up. Figure 30 indicates the performance of the reactor. The right – hand side of the graph represents the volumetric productivity, expressed in L CH₄ L_{reactor}⁻¹ with the plot line with symbols. The left – hand side corresponds to the methane composition (%) and is illustrated by bars. The average value is given along with standard deviation.

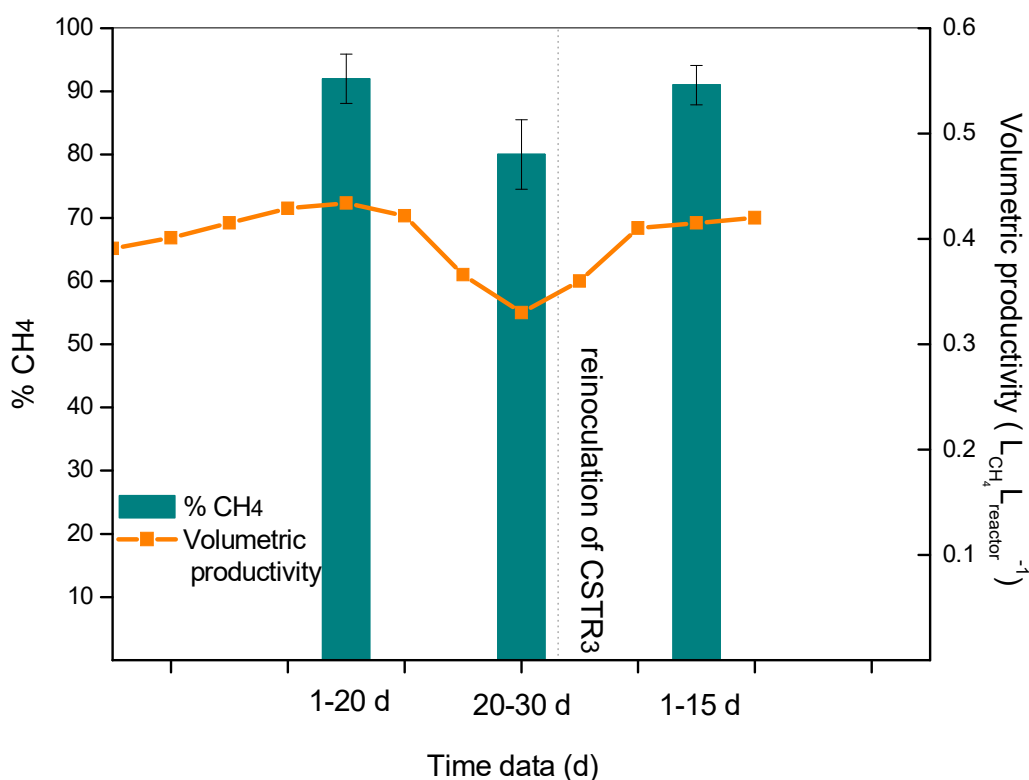


Figure 30: Methane composition and volumetric productivity of CSTR₃.

Initially the volumetric productivity and the methane composition were higher. The average values were $0.43 \pm 0.13 \text{ L CH}_4 \text{ L}_{\text{reactor}}^{-1}$ and $92.4 \pm 1.1\%$, respectively. This could be due to the remaining organic content in the inoculum. Another reason contributing to the excess CO₂ and CH₄ production may be the bicarbonate in the inoculum influencing the distribution of carbon dioxide ($\text{CO}_2 \leftrightarrow \text{HCO}_3^-$) between gas and liquid phase.

After that, methane content reached a value as high as 94.8%, with hydrogen and carbon dioxide only making up a small amount of the biogas (2.1% H₂ and 5.5% CO₂). pH had an average value of 7.8, below the inhibition threshold of 8.0.

Volatile fatty acids were also determined. Acetate was the main metabolite detected in the liquid phase, with a concentration between 0.5 and 0.6 g L⁻¹ while the rest of volatile fatty acids had a concentration < 0.3 g L⁻¹. Acetate had the highest concentration among the other VFAs also in the used inoculum. That played a role in the reason on the prevalence of acetate. Yet, there is another pathway, in which

acetate is produced, to be taken under consideration. The alternative pathway is homoacetogenesis and is presented in Figure 31.

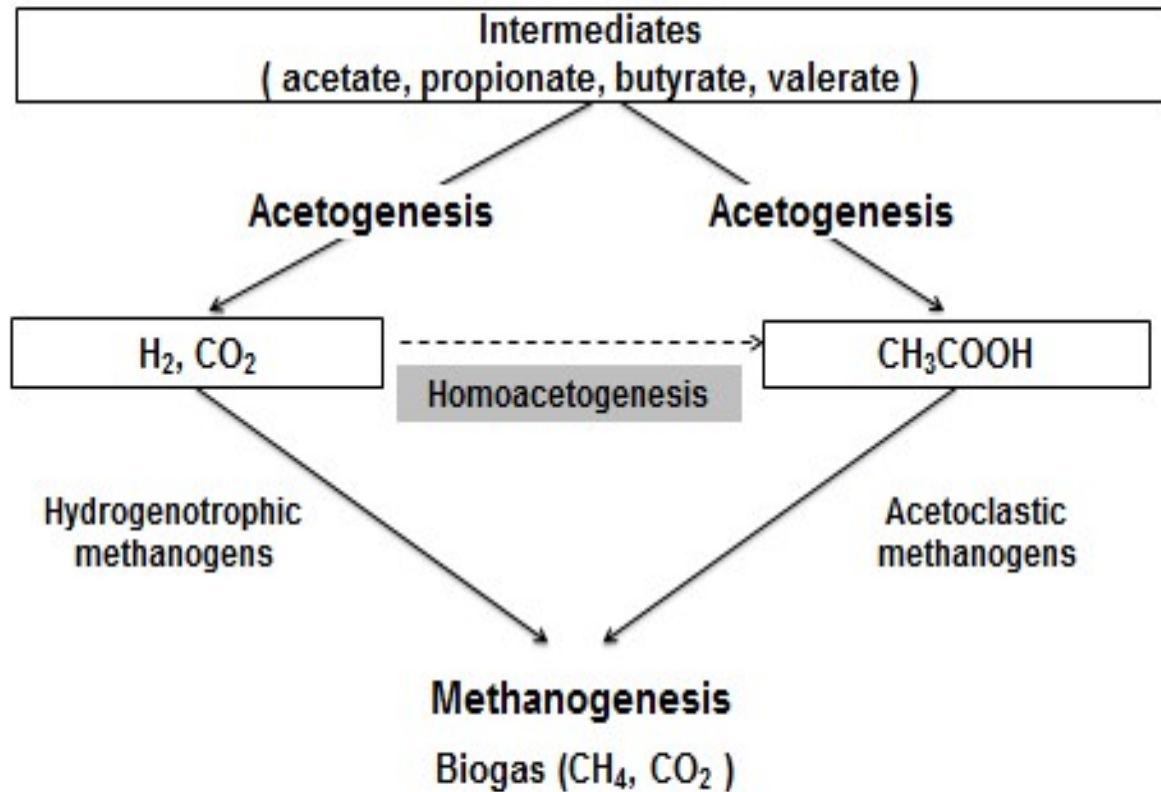


Figure 31: Homoacetogenesis metabolic pathway.

Via homoacetogenesis some H₂ and CO₂ can be converted to acetate. Then acetate can be further converted to CH₄ and CO₂ by acetoclastic methanogens (Demirel 2008).

After about 22 – 23 days of operation, which corresponded to around 1.2 times the HRT of the CSTR₂ system, the values start significantly decreasing. CH₄ content fell off to values under 85%. Volumetric productivity as well was decreased to values < 0.35 L CH₄ L_{reactor}⁻¹. The decline of the system kept further. The reason for the failure of the system was expected. According to biological methanation reaction, apart from the production of CH₄, water is also produced. During the *in – situ* continuous experimental period this did not affect the system in any way. Nonetheless, when there is no liquid substrate entering the system and subsequently replacing the

organic material as well as the microorganisms, inoculum ages. In conjunction with that organic material, trace elements and microbes get depleted.

After around one month of operation inoculum was replaced with fresh one. The performance of the system was restored giving similar results. Furthermore, the re – inoculation of the reactor presented the advantages of the residual organic substrate along with the presence of new active methanogens.

Finally, the possibility of starting the liquid substrate feeding stream again conjointly with hydrogen injection was evaluated.

As a result, CSTR₃, after 15 days of the re-inoculation of the system, process conditions were changed to the same as those in CSTR₂. The acclimatization period lasted longer than before but the system exhibited the same process efficiency as previously reported (pH: ~ 7.8, CH₄: ~ 89 %, CH₄ yield: 0.354 L CH₄ g VS_{fed}⁻¹).

5.7 Anaerobic digestion pathway through FISH analysis

The composition of the methanogenic communities present in the anaerobic system was studied with fluorescence in situ hybridization (FISH) analysis. Samples were obtained from both experimental systems. The assessment of identification of microbial groups with the use of order – level probes was carried out.

Microorganisms mediating acetotrophic and hydrogenotrophic methanogenesis mainly belong to five phylogenetic orders (Garcia et al., 2000). The main methanogenic orders and their characteristics are shown in Table 13 (Karakashev et al., 2005, Adam et al., 2006). The hydrogenotrophic order of *Methanopyrales* was excluded from this assessment since it is consisted of hyperthermophilic member species.

Table 13: Main characteristics of methanogenic order.

Phylogenetic order	Cell morphology	Characteristics
<i>Methanomicrobiales</i>	Small rods, irregular cocci, curved rods	Hydrogenotrophic, 0 – 60° C
<i>Methanobacteriales</i>	Rods or filaments	Hydrogenotrophic, 15 – 97° C
<i>Methanococcales</i>	Irregular cocci	Hydrogenotrophic, 18 – 94° C
<i>Methanosarcinales</i>		2 – 70° C
<i>Methanosaetaceae</i> family:	Rods or filaments	Strictly acetoclastic
<i>Methanosarcinaceae</i> family:	Irregular cocci	Acetoclastic or hydrogenotrophic

The FISH analysis of Nielsen et al., 2009 protocol was applied to reactor samples. The probes used as well as their target orders or families are presented in Table 14. EUB338 and ARC915 were used to identify most members of *Bacteria* and *Archaea* respectively. Probes data was taken from <http://www.probeBase.net>. The probes were labeled with either Cy-3, a fluorescent cyanine dye or 6-Fam (6 - carboxyfluorescein). The absorption wavelength and the emission wavelength for Cy-3 are 550 nm and 570 nm. While for 6-fam dye is 494 nm and 520 nm, respectively.

Table 14: Oligonucleotic probes used and their specificity.

Probe	Phylogenic group /Specificity	Probe sequence (5' – 3')	Formamide (%)	Label	Reference
EUB338	most Bacteria	GCTGCCTCCCGTAGGAGT	0 – 50	Cy-3 6-Fam	Amann et al. 1990
ARC915	Archaea	GTGCTCCCCCGCCAATCCCT	0 – 50	Cy-3 6-Fam	Stahl and Amann,1991
MX825	Methanosaetaceae / Methanosaeta spp.	TCGCACCGTGGCCGACACCTACG	50	Cy-3	Raskin et al. 1994
MS1414	Methanosarcinaceae / Methanosarcina spp., Methanococcoides spp., Methanlobus spp., Methanohalophilus spp.	CTCACCCATACCTCACTCGGG	50	Cy-3	Raskin et al. 1994
MG1200	Methanomicrobiales / Methanomicrobium spp., Methanogenium spp., Methanoculleus spp., Methanospirillum spp., Methanocorpusculum spp., Methanoplanus spp.	CGGATAATTCGGGGCATGCTG	20	Cy-3	Sekiguchi et al.1999 Raskin et al. 1994
MB1174	Methanobacteriales / Methanobacterium spp., Methanobrevibacter spp., Methanosphaera spp.	TACCGTCGTCCACTCCTTCCTC	45	Cy-3	Crocetti et al. 2006
MC1109	Methanococcales / Methanococcaceae	GCAACATAGGGCACGGGTCT	45	Cy-3	Raskin et al. 1994
MSMX860	Methanosarcinaceae / Methanosarcina spp., Methanococcoides spp., Methanlobus spp., Methanohalophilus spp., Methanosaeta spp.	GGCTCGCTTCACGGCTTCCT	45	Cy-3	Crocetti et al. 2006 Raskin et al. 1994

Samples from different operational phases were evaluated. Concentration of volatile fatty acids (VFA), biogas production rate, organic loading rate (OLR) and biogas recirculation appeared to have no influence on the methanogens presence.

Samples contained a high solids concentration. Thus, dilution prior to FISH analysis was necessary. Artifacts were present making the assessment process difficult. The following Figure 32 indicates the typical morphology of the detected phylogenetic groups. Probes MC1109 and MG1200 are not given in figures. Typical morphology of these methanogens was not observed leading to the conclusion that Methanococcales and Methanomicrobiales group were not mediating the methanogenesis.

Regarding the rest of the probes, initially ARCH915 and EUB338 were combined. A strong presence of Archaea with cell morphology of filaments was detected.

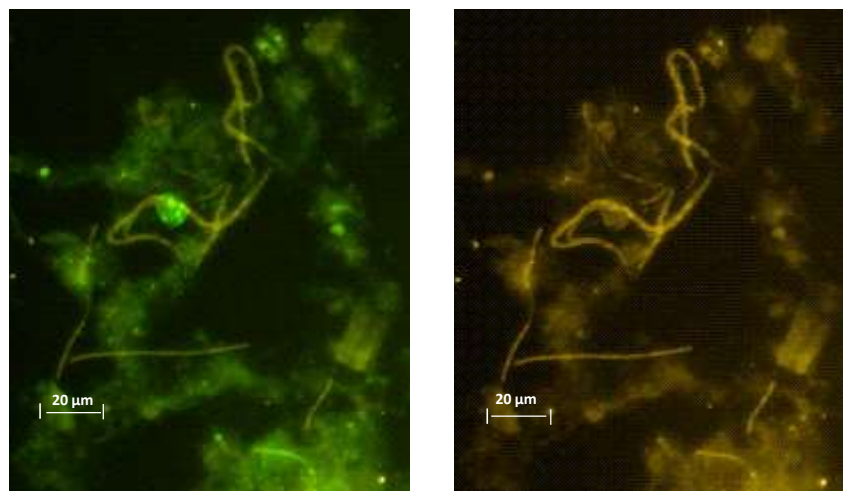


Figure 32: Combination of probes ARCH915 (Cy-3) and EUB338 (6-FAM). Left side: merged channels, Right side: detected Archaea.

In order to further investigate the active methanogens into the anaerobic reactor each probe mentioned in Table 14 was combined with the probe ARCH915. The formamide concentration was decided based on the supplier's references.

Probes ARC915 and MSMX860 were combined. With the MSMX860 specific probe, which detects the order Methanosarcinales, the existence of acetotrophic methanogenic Methanosaetaceae family was verified (Figure 33).

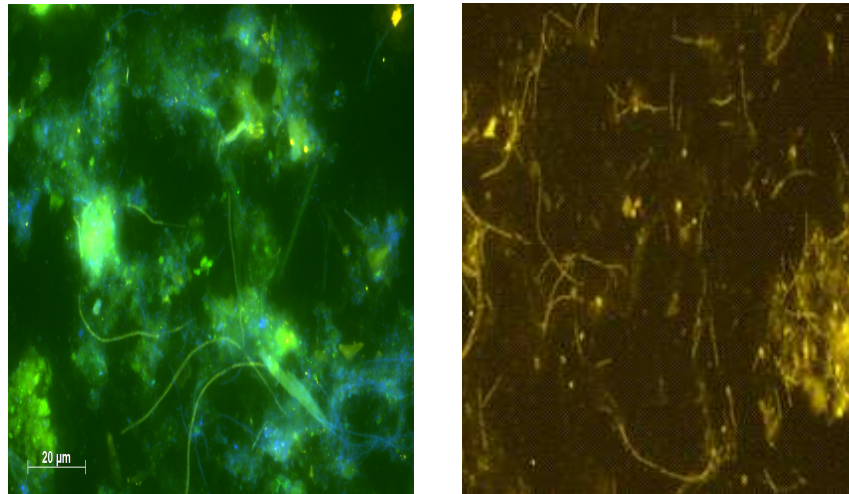


Figure 33: Left side: Typical morphology of Methanosaetaceae (filaments) detected with probe ARCH915 (6-FAM) and MSMX860 (Cy-3), Right side: Typical morphology of Methanobacteriaceae (filamentous rods) detected with probe MB1174 (Cy-3).

With the use of MB1174 probe Methanobacteriaceae family was detected which generally grow and produce CH_4 by reducing CO_2 with hydrogen.

According to Karakashev et al., (2005) in sewage sludge digesters the most frequently observed hydrogen utilizers are members of the Methanobacteriales. Furthermore, the results of the present study are in accordance with previous studies (Sekiguchi et al., 1999, McMahon et al., 2001) reporting the dominance of the Methanosaetaceae in sludge digesters.

Fluorescence in situ hybridization analysis during the continuous experiments indicated that methanogenesis was performed by both acetotrophic and hydrogenotrophic pathway.

5.8 Comparison of performance of all experimental configurations

During this present work three experimental configurations were implemented and evaluated. Specifically anaerobic treatment of sewage sludge was tested as well as *in – situ* and *ex – situ* biogas upgrading with the use of hydrogen.

CSTR₁ was a continuous system treating sewage sludge. Experiments were conducted in mesophilic temperature. The stability and efficiency of the system was investigated. Gradually decreased hydraulic retention time was implemented. Through this operation optimum conditions were determined. The average methane content achieved was 68.7%. Biogas production rate was 0.467 L L_{react.}⁻¹ d⁻¹. The obtained methane yield was 256 LCH₄ kg⁻¹VS. Finally, biogas recirculation was also evaluated. Both biogas and methane production rates were increased only by a 2 to 3%. After that, system CSTR₁ was employed as a control reactor for the second experimental system.

During the continuous operation of system CSTR₂ *in – situ* biogas upgrading was implemented. After an initial pre – H₂ phase for adjustment reasons, hydrogen was added into the reactor. Different hydrogen flow rates were tested. The optimum flow rate was in accordance to the stoichiometric ratio of H₂:CO₂ for CH₄ production given by the Sabatier's reaction (see Reaction 6). Methane production rate was around 16.3% higher compared to the control reactor. Produced biogas had an average composition of 80.3% CH₄. CO₂ was reduced to 15.5%. Hydrogen was not fully consumed.

As Figure 34 illustrates the methane production rate, given in LCH₄ kg⁻¹VS, is compared between the methane yield of the theoretical yield (BMP_{th}), the biomethane potential test (BMP), the control reactor (Control) and the CSTR₂ system.

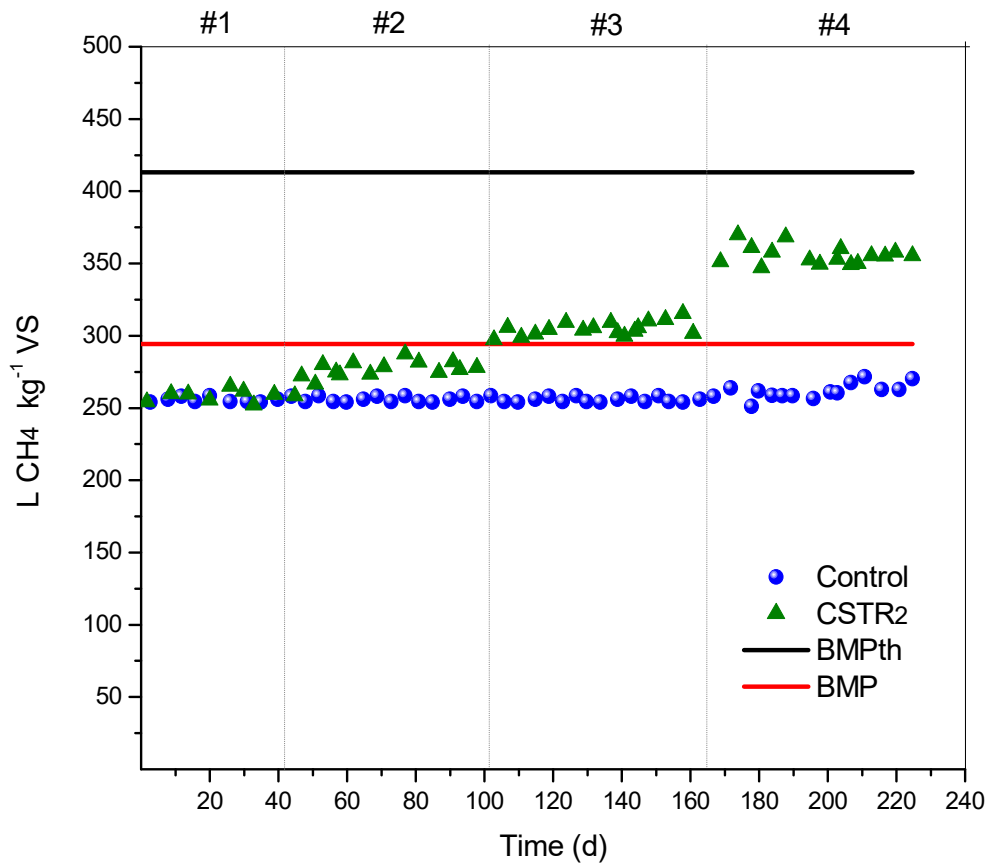


Figure 34: Comparison of methane production rate of the theoretical yield (BMP_{th}), the biomethane potential test (BMP), the control reactor (Control) and the CSTR₂ system.

Regarding the theoretical calculation of maximum methane potential (BMP_{th}) and methane concentration it was evaluated by using Buswell Equation (see Equation 10). This formula assumes that the feedstock is consisted of C, O and H. The contribution of nitrogen (N) and sulphur (S) is considered to be negligible. Moreover, since neither non-degradable material nor the energy demand of the microbes is considered, it is not a surprise that the value obtained by the Buswell Equation (431.2 in LCH₄ kg⁻¹VS) is the higher than that obtained in BMP assays or reactor studies.

The BMP assay, was carried out by the Bioprocess™ automatic methane potential test system (AMPTS II, Bioprocess Control AB, Sweden), reduce demerits of Buswell calculations. Methane production is directly measured on-line via liquid displacement and buoyancy method. Hence, the methane yield approaches the real conditions and values. The BMP results reached 71.2% of the theoretical biomethane potential with a value of 294.2 LCH₄ kg⁻¹VS.

Regarding the control reactor and the first phase of CSTR₂, where in both cases no hydrogen was used, the methane yield was 256 and 259 LCH₄ kg⁻¹VS, respectively. These values correspond to 88% of the BMP_{th} yield. During operational phase 4#, where biogas recirculation was implemented, the value for the control reactor is slightly higher.

When hydrogen was injected into the anaerobic reactor (CSTR₂) the scenery was quite different. When hydrogen flow rate corresponded to 3 times the volume of CO₂ production rate in the control reactor (phase #2 in figure 34), methane production rate did not reach the BMP results but it was around 94.1 % of its' value.

During phase #3 as shown in Figure 34, hydrogen utilization by microorganisms resulted not only in a higher value but actually methane yield for CSTR₂ overcame the BMP value. It reached a rate of 304 LCH₄ kg⁻¹VS. This result occurred when hydrogen flow rate inserted to the system was increased. It was changed to a value corresponding to 4 times the volume of CO₂ production rate in the control reactor. This was in accordance to the stoichiometric ratio of H₂:CO₂ for CH₄ production given by the Sabatier's reaction (see Reaction 6).

Finally, during the last phase, biogas recirculation was implemented. The system gave the highest performance yield. Regarding biogas composition, CH₄ resulted in an average of 89% with a maximum of 95.2%. With a methane production rate of 356 LCH₄ kg⁻¹VS the system demonstrated a yield 21% higher than the BMP assay and it reached a high percentage (82.5%) of the maximum theoretical potential.

In order to evaluate other parameters as well, a comparison table (Table 15) including all three continuous systems is given. The selected operational phases correspond to the optimum applied conditions where biogas recirculation was implemented.

Table 15: Average values of the main parameters for all three experimental configurations.

Parameter	CSTR ₁ (control)	CSTR ₂ (<i>in - situ</i>)	CSTR ₃ (<i>ex - situ</i>)
Operational phase:	#6 HRT = 19.6 d no H ₂ injection biogas recirculation	#4 HRT = 19.6 d H ₂ injection H ₂ :CO ₂ = 4:1 biogas recirculation	1-22 days H ₂ and CO ₂ injection H ₂ :CO ₂ = 4:1 biogas recirculation
Biogas production rate (L L ⁻¹ d ⁻¹)	0.467 ± 0.012	0.499 ± 0.014	0.443 ± 0.018
Biogas composition			
CH ₄ (%)	68.7 ± 0.8	89.1 ± 2.3	92.4 ± 1.1
CO ₂ (%)	31.3 ± 0.8	8.4 ± 1.6	5.5 ± 0.3
H ₂ (%)	–	2.5 ± 1.1	2.1 ± 0.7
CH ₄ production rate (L L ⁻¹ d ⁻¹)	0.321 ± 0.01	0.445 ± 0.02	0.409 ± 0.02
CO ₂ production rate (L L ⁻¹ d ⁻¹)	0.146 ± 0.01	0.042 ± 0.01	0.023 ± 0.01
H ₂ flow rate (L L ⁻¹ d ⁻¹)	–	~ 0.56 – 0.58	~ 0.58
Biogas yield (L _{biogas} gVS _{fed} ⁻¹)	0.372 ± 0.01	0.398 ± 0.005	–
CH ₄ yield (L _{CH₄} gVS _{fed} ⁻¹)	0.256 ± 0.01	0.356 ± 0.007	–
pH	7.27 ± 0.12	7.85 ± 0.13	7.8 ± 0.08

As table 15 indicates both *in – situ* and *ex – situ* hydrogen utilization resulted in higher methane content. Biogas production rate was about 7% higher and CH₄ production rate more than 30% than the control reactor. During *ex – situ* experiments biogas production rate is lower. The lack of substrate feeding into this system (CSTR₃) reflects the lack of volatile solids. Hence, a reduced amount was converted to biogas.

The addition of hydrogen into the system resulted in an increase of pH. For the control reactor the average pH value was 7.27. Hydrogen addition led the pH values of CSTR₂ to increase. The average value was 7.85 but neither process inhibition nor reduction of H₂ and CO₂ conversion to methane was noticed. System CSTR₃ presented also pH values close to 7.8.

Hydrogen was converted to CH_4 . Composition of biogas is also presented at Figure 35. *In – situ* biogas system (CSTR_2) resulted in an average of 89% with a maximum of 95.2%. While at the control reactor the average value was 68.7%.

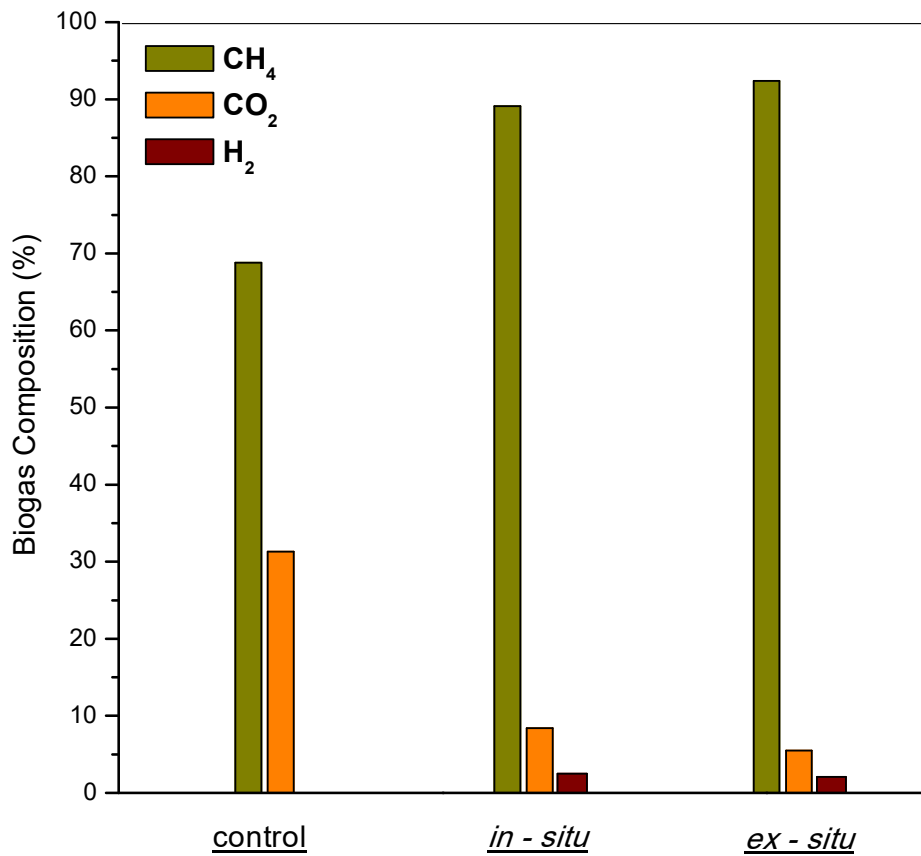


Figure 35: Average value of the main biogas components for all three experimental configurations.

For the *ex – situ* biogas system (CSTR_3) the average value of methane was 92.4% with a maximum of 94.8%. However, after about 22 – 23 days of operation, it was decreased to values under 85%. Biogas volumetric productivity was decreased as well. Hence, re-inoculation of the system was required.

In comparison to the control reactor, hydrogen and carbon dioxide were present in smaller amounts in the biogas produced by both *in – situ* and *ex – situ* systems (8.4% H_2 , 2.5% CO_2 and 2.1%, 5.5% CO_2 , respectively).

5.9 Comparison of performance with other reported systems

Regarding biological methanation a numerous reactor configurations have been trialed at lab – scale. Besides different layout of the reactor, other parameters such as type of waste, temperature, mixing velocity and gas flow rates were evaluated.

Various systems for biological methanation through coupling of H_2 with CO_2 and performance data are presented in Table 16.

Luo & Angelidaki (2012) investigated the effect of the mixing intensity on the efficiency of the process. They have presented an *ex-situ* biogas upgrading method. In a separate biogas reactor biogas and hydrogen were fed into the system. The reactor contained enriched hydrogenotrophic methanogens and the continuous experiment was conducted under thermophilic temperature ($55^\circ C$). By decreasing the mixing speed the CH_4 content was increased to around 95%.

In – situ biogas upgrading system treating cattle manure was also evaluated by Luo et al., 2011. Hydrogen was added to the system through ceramic diffusers. It was entering the liquid phase distributed into small bubbles for better contact with the liquid. The reactor was operated under thermophilic conditions ($55^\circ C$). While the carbon dioxide content was only 15%, the pH was increased to levels higher than 8. Hence, slight inhibition of methanogenesis was reported.

For the maintaining the pH between 7 and 8 a co – digestion of manure and acidic whey was tested (Luo & Angelidaki, 2013b). A column diffuser was used apart from ceramic one. So the hydrogen distribution system and the mixing intensity were evaluated. The best biogas composition obtained had 75% of CH_4 and pH values were kept below 8 preventing any process inhibition.

Insertion of hydrogen by a hollow fiber membrane (HFM) (Luo & Angelidaki, 2013a) was also trialed. Bubbleless gas transfer through the HFM module was used for *in – situ* biogas upgrading. A mixture of cattle manure and whey was used as a substrate. Gradually increased hydrogen flow rates resulted in higher CH_4 content with a maximum of 96.1%. The study demonstrated the biofilm's contribution to the consumption of hydrogen.

Burkhardt et al., (2013, 2015) reported an improved liquid mass transfer using a trickle-bed reactor. Larger transfer surface was available providing longer retention times for the process to be carried out. Due to the formation of a three-phase system on the carrier surface methane concentrations achieved were higher than 97%. Both continuous and batch tests were carried out.

Bassani et al., (2015) implemented a two stage setup composed by two serial-connected continuous stirred-tank reactors (CSTRs), treating cattle manure. Biogas was produced in the first reactor, whereas the second one, where hydrogen was injected, was treating the effluent serving as upgrading chamber. The configuration resulted in an average CH₄ content of 89%.

Besides continuous stirred-tank reactors (CSTRs) some different types of reactors were operated.

Lee et al., (2012) reported a system composed of an up-flow anaerobic fixed bed reactor. The fixed bed reactor was packed with reticulated polyester urethane sponge. The sponge acted as a support for biofilm growth and to let gas bubbles stay longer in the reactor. The authors concluded in the fact that the specific reactor type, when employing hydrogentrophic methanogens, can achieve 100% conversion rate of carbon dioxide and hydrogen at 3.8 h retention time.

As an alternative, an upflow anaerobic sludge blanket (UASB) reactor was also tested (Bassani et al., 2016). The granular reactor was connected to a separate chamber where H₂ was injected. Alumina ceramic sponge and rashig rings both were tested as packing materials. In that case, CH₄ content increased up to 82% by using a metallic diffuser followed by a ceramic sponge along with a mild gas recirculation.

Finally, the operation of *ex – situ* biological methanation at two thermophilic temperatures (55° C and 65° C) was investigated in batch closed systems by Guneratnama et al., 2017. Use mixed culture and enriched culture, with different retention times, with H₂ and CO₂ as the influent gases was tested. Methane content in excess of 90% was achieved and the importance of re - inoculating the reactor was highlighted.

Most of the available literature on biological methanation investigates agricultural substrates. According to European Biogas Association, Germany by the end of 2014 had the most biogas plants in Europe. The 8,005 biogas plants in the agriculture sector make the biggest contribution to biogas production today with electricity and heat supplies (Svensson and Baxter, 2016). However, a high proportion of biogas produced in anaerobic digestion plants is from those on municipal sites of wastewater treatment plants (WWTP) (Bachman, 2015). In Germany the number of biogas plants treating sewage sludge from WWTP covers 13% of the total number of biogas plants corresponding to 7% of the energy production (data from 2015) (Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety, 2016).

Thus the present study aimed to assess biological methanation process on a continuously stirred tank reactor treating sewage sludge. Both *in – situ* and *ex – situ* biogas upgrading with the use of hydrogen were evaluated.

CH₄ content in the produced biogas resulted in an average of approximately 89%, with a maximum of 95.2% for *in – situ* biogas upgrading.

Additionally, the process stability was tested also while having a gaseous substrate as a sole feeding. The system corresponded well, no pH control was required. Carbon dioxide and hydrogen were highly consumed giving a 94% of CH₄ content. Therefore it becomes clear that in case of lack of liquid substrate the system can correspond well but the re-inoculation of the reactor after about 22 – 23 days is a prerequisite.

Finally, the stability of the process by the alteration of a system from *in – situ* to *ex – situ* upgrading method and vice versa was tested.

Table 16: Reported systems and performance data.

Reactor	Temp. (°C)	Inoculum	Substrate	Influent gas	Operation	Maximum methane concentration	References
CSTR	55	Digested manure	Cattle manure	H ₂	continuous	65	Luo et al, 2011
Trickle-bed	37	Digested sewage sludge	–	H ₂ :CO ₂	batch + continuous	96	Burkhardt et al 2013,2015
CSTR	55	Digested manure	–	H ₂ :CH ₄ :CO ₂	continuous	95.4	Luo & Angelidaki 2012
CSTR - HFM	55	Digested manure	Cattle manure, whey	H ₂	continuous	96.1	Luo & Angelidaki 2013a
CSTR	55	Digested manure	–	H ₂	continuous	75	Luo & Angelidaki 2013b
Upflow anaerobic fixed bed	35	Digested sewage sludge	–	H ₂ :CO ₂	continuous	n.s	Lee et al, 2012
Two-stage CSTR	35/55	Animal slurry, food waste	Cattle manure	H ₂	continuous	92	Bassani et al, 2015
UASB	55	Granular sludge	Potato starch wastewater	H ₂	continuous	82	Bassani et al, 2016
Closed batch systems	55/65	Digested maize, grass farmyard manure	–	H ₂ :CO ₂	batch	92	Guneratnam et al. 2017
CSTR	37	Digested sewage sludge	Sewage sludge	H ₂ H ₂ :CO ₂	continuous	95.2 94.8	Present study

6. Concluding remarks and outlook

Use of the Power to Gas concept at a biogas facility has great potential. When excess electricity is available, surplus electricity may be stored through the power to gas concept by producing hydrogen from electrolysis.

The hydrogen can thereafter be combined with CO₂ to produce methane in a number of ways. Hydrogen is added to the anaerobic reactor and reacts with raw biogas within the biogas digester. In this *in – situ* biological methanation process it is hard for biomethane standard suitable for gas grid injection or for vehicle use that will be constantly achieved. Thus, a smaller biogas upgrading step will be required if biomethane is the proposed end product.

Methanation may also take place with the use of the biogas upgrading unit. This could be employed at a site where biogas upgrading is already in place and a very concentrated CO₂ stream is available. CO₂ can enter the reactor along with the H₂ coming from electrolysis. The CO₂ can either be of fossil or renewable origin, extracted from the air or various industrial waste gases.

In the present work, biological methanation with hydrogen utilization in the anaerobic digestion process was evaluated. The supply of hydrogen to an anaerobic digester for *in – situ* biogas upgrading as well as implementation of an *ex – situ* biogas upgrading process for hydrogen and carbon dioxide utilization, as the sole feeding stream, were examined.

Three experimental systems were installed and operated in a continuous way.

Initially, a stable process of treating sewage sludge for the production of biogas was designed and operated as the control reason. The treatment system's performance was monitored.

In – situ biogas upgrading was then installed and operated by introducing directly H₂ to the organic waste feeding stream of a CSTR reactor. Various operational conditions were implemented and the system's response in the application of each operational phase was evaluated. The effects of different H₂ flow rates on the process performance were monitored. High conversion efficiency was obtained. CH₄

production rate with H₂ utilization was increased. For further improving the CH₄ yield biogas recirculation was tested. CH₄ production rate with H₂ utilization was increased by more than 30% compared to the control reactor. CH₄ content resulted in an average of approximately 89%, with a maximum of 95.2%.

Fluorescence in situ hybridization analysis verified that methanogenesis was performed by both hydrogenotrophic and acetotrophic methanogenic pathway.

Furthermore, implementation of an *ex-situ* biogas upgrading process for hydrogen and carbon dioxide utilization, independent on biomass availability, was evaluated. During this operation the feeding gas mixture consisted of 80%:20% hydrogen and carbon dioxide, respectively. The gas injection rate was set based on the control reactor's biogas production and composition. The average value of methane was 92.4% with a maximum of 94.8%. However, after about 22 – 23 days of operation, it was decreased to values under 85%. Biogas volumetric productivity was decreased as well. Hence, re-inoculation of the system was required.

According to biological methanation reaction, apart from the production of CH₄, water is also produced. Also, since there is no liquid substrate entering the system and subsequently replacing the organic material as well as the microorganisms, inoculum ages. Due to the combination of these two elements, organic material, trace elements and microbes get depleted.

Therefore, there is a large scope for further research in order to develop, improve and optimize an efficient system with respect to the re – inoculation downside.

In conclusion, there are a number of questions to be answered when combining a biogas plant with a power to methane application. The answer of these questions can not be definitive because the industry is still developing and the answer depends on variables including: the size of the facility, whether it is a new facility or an addition to an existing facility, if an existing facility whether the biogas is used in CHP or upgraded, the policy and tariffs of the country of operation.

Yet, biological methanation can contribute to the net increase of CH₄ production and to the conversion of the excessive renewable energy into a storable gas. The main benefit will be the possible use of biogas as an alternative to natural gas and the use

of the existing infrastructure system for storing electricity that otherwise would be curtailed.

Overall, biological methanation can contribute for a sustainable beneficial Power to Gas model.

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